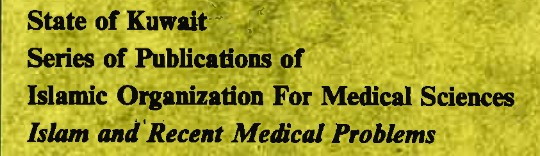




State of Kuwait
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Islamic Organization For Medical Sciences
Islam and Recent Medical Problems





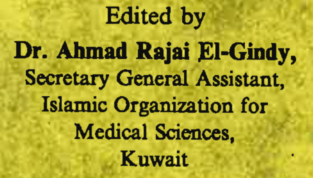
PHARMACOLOGICAL AND CLINICAL EVALUATION

OF

Some Plants Used in Islamic Medicine



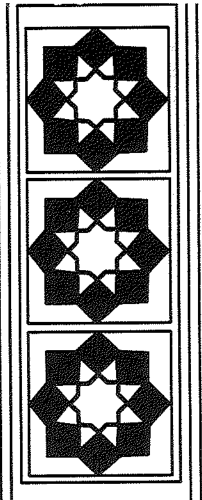
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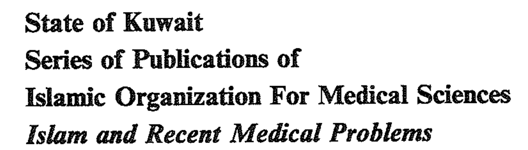


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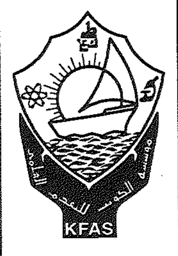


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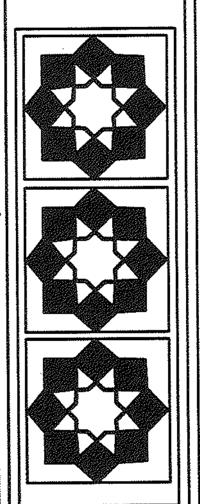
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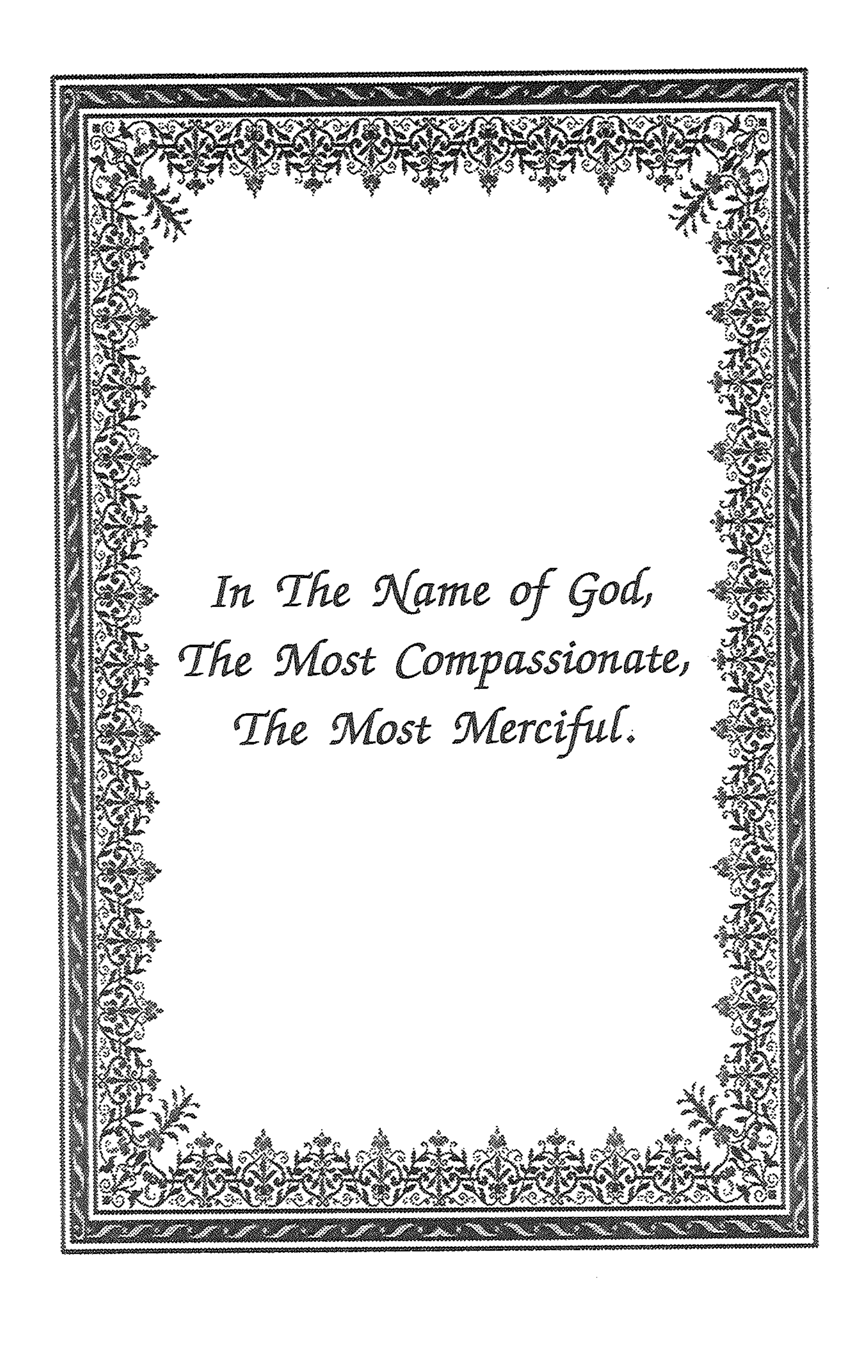
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In The Name of God,
The Most Compassionate,
The Most Merciful.

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PREFACE

Dr. AbdulRahman A. Al-Awadi
President
Islamic Organization for Medical Sciences
KUWAIT

Thanks to Allah Almighty for guiding us to Islam, enlightening our hearts with true belief, discarding all grief, dispelled worries, and freed our homeland.

This series comes following fifteen years of the idea of establishing the Islamic Organization for Medical Sciences and after its participation in local and regional book exhibitions where our volumes of Islamic Medicine were greatly appreciated by the visitors. However, because of the soaring cost of paper and publication, the individual book keeping has become very difficult, especially in the non-Gulf Arabic and Islamic countries, as bread earning receives the first priority of the inhabitants of these countries. Keeping in view the fact that the individuals need to be informed, and educated, of the important matter to make them effective member of their community and also a messenger to other communities, it is vital to provide them the contents of these conferences in a simplified way to enable them to carry along and comprehend the scientific purport.

In order to facilitate the possession of these books by the individuals, the Islamic Organization for Medical Sciences has decided to issue a series of publications under the title "The Cultural Series of the Islamic Organization for Medical Sciences". Although the Organization is shouldering the largest share of the cost of production and publication of these books, still these are out of reach of a large section of Muslim individuals, due to escalating

cost of living. The great sum of money available to the Organization is spent in bringing together and collecting the prominent thinkers of our Islamic nation in order to achieve appropriate opinions and covisions of the Islamic Scientists about right topics that need insight and the true objective word. And, subsequently, to present this information to every individual willing to increase his/her knowledge about the doctrinal writings in scientific medicine, as this prominent group of writers/thinkers sees this as an ordinance and a religious obligation to provide for all the Muslims, and to disseminate the message to the largest number of the people of this nation.

This series will include a group of books, each dealing with specific topic, as collected from the articles written under the respective domains and previously published in the Proceedings of the Islamic Medicine Conferences held under the auspices of the Organization. Moreover, all these publications shall remain concerned with one vital topic, that is, the Islamic Medicine. By doing so, we hope to have shouldered the burden off the Arabic/ Islamic reader to enable him/her to own the right material and hoping to have clarified a lot of mystery about the subject of Islamic Medicine to the Muslim and Arab readers.

Herein, I beseech Allah to guide our steps to what He likes and approves of.

INTRODUCTION

Dr. Ahmad Rajai El-Gindy
Secretary General Assistant
Islamic Organization for Medical Sciences,
KUWAIT

Thanks to Allah, the Almighty; the thanks of the grateful, the obedient, and the desirous of His forgiveness and retribution, beseeching him, to guide us to the right deeds, with praying and blessing his illiterate prophet (ﷺ) who said,

"When Adam's son dies, everything is separated from him except for three things, a current charitable deed, a righteous boy praying for him, and a useful science."

We pray to Allah that these series of publications will be of scientific use to the Muslims in particular, and to humanity in general.

This introduction will be included in all the publications of this series in order to acquaint the reader, who wishes to acquire one or more parts of it, with the objectives of the Organization, and the reasons behind its being established. We wanted to put down these words to the readers concerned about what we did, while the second part of this introduction will be specifically written for each book, including a summary of the researches therein.

Since the emergence of the idea of the Islamic Medicine fifteen years ago, the discussion of the meaning of "ISLAMIC MEDICINE" did not stop; the people argued: Is there an "Islamic" and "a non-Islamic" Medicine? and we found ourselves in front of three opinions-

The first opinion:-Medicine is a human heritage; inherited successively by generations, and it is a human experience, acquired by technical and scientific practice, and religion has no role in it,

and there is no need to indulge Islam in this subject to protect it from human practices.

The second opinion:- Islamic medicine means nothing to them except it is a past heritage, and we do not need it now because the world is talking about organs transplantation, genetic engineering, Lazer beams... etc. They even considered it a call of underdevelopment, and we have to put it behind closed doors; those are who don't want Islam to be mentioned at all.

The third opinion:- Although medicine is human practices and experiences, but every religion and every heavenly message has its own nature, ethics and practices which are derived from its teachings, and which adds to it its own style. The Islamic era was characterized with a comprehensive change in both the concepts and practices of the people; these concepts and practices were derived from the Holy Quran and the honored *Sunna*, and were followed by the Orthodox Caliphs, which produced a good harvest, with which they ruled the world, east and west with a civilization - Man was its master, good science its way and the strong belief its pillars. This civilization lasted for five complete centuries, and it was never stingy with its knowledge and arts on humanity.

For there is no favor of an Arab on a Persian, nor of a white man on a black man except by piety and good deeds, this was said by the enemies before the friends; and (Sarton's) testimony in his encyclopedia, the history of sciences, is the best evidence; (Sarton) divided the world into eras of civilizations like the Pharonic, the Babylonian, the Somarian, the Chinese, the Greek, then the Islamic Civilization which flourished in all walks of Arts and Sciences for five consecutive centuries, and in it were eminent scientists, thinkers, philosophers, physicians, pharmacists, engineers, algebraists, astronomers, agriculturists, and people of thoughts who were distinguished with their excellence in the Divine Law, besides the cosmetic sciences.

To all these we say, our view of this topic is derived from Islamic Law, which came with its five goals, which are sustaining the religion, the mind, the self, the honor and the wealth. If we studied these goals, we'll find that three of them are concerned with Man's well being; that is the mind, the self and the honor, as for the other two, they are concerned with man's health, as there is no keeping of religion, nor of wealth without a strong good Muslim (The best one to hire is the strong and honorable). The prophet (ﷺ) defined three main points, if provided in any MAN, he will lead a very happy life, as he (ﷺ) says

"The one who sleeps secured in his bed, healthy in body, well provided for his day's food, ... he is like the one who owned the entire world."

In other words, he has got social, health and psychological security. Thus the Islamic Law talks about well being in its widest range. "The strong believer is more loved by Allah than the weak one, and both are good." The Islamic Law did not speak about medicine in its narrow sense, through which the others are trying to attack us, but medicine is the means of health, and Al-Ghazaly, a Muslim religious leader, considered medicine as a religious ordinance in all Muslim homes.

Islam considers enjoying a good health one of the biggest blessings of Allah; as mentioned in the wise saying of the prophet (ﷺ), "*Two blessings many people are not endowed with; health and leisure time*". These two blessings are two of the very important duties that must be kept by man as the Islamic rule says, "Whatever is not perfect without a duty, is itself a duty", thus man is not allowed to neglect his health, as it should not be neglected, because this is considered an aggression on the whole nation as it is so mentioned in the Holy Quran:-

"FOR THAT ACCOUNT WE ORDAINED FOR THE CHILDREN OF ISRAEL THAT IF ANY ONE SLEW A PERSON -

UNLESS IT BE FOR MURDER OR FOR SPREADING MISCHIEF IN THE LAND - IT WOULD BE AS IF HE SLEW THE WHOLE PEOPLE, AND IF ANY ONE SAVED A LIFE, IT WOULD BE AS IF HE SAVED THE LIFE OF THE WHOLE PEOPLE"

(Al-Maeeda: 32).

Abu-Bakr, (رضي الله عنه) said "I heard the prophet of Allah (ﷺ), saying, *"Ask Allah for certainty and health, for they are the best blessings bestowed on man is being healthy after being certain"*; thus self-relief is the true gate to health; either psychological or bodily health, their only true gate is strong belief, belief in slavery to Allah, whatever inflicts you was not to wrong you, and whatever to wrong you, was not to inflict you.

The belief in the acts of worship which are prescribed by Islam are:-

Prayer is secret talk with Allah Almighty, and self purification five times a day standing in front of the Creator,

Fasting is self restrain from evil desires, and true feeling of the hunger of the Muslim brother who is deprived of a morsel of bread,

Zakat or *Alms* is a sacrifice, self cleanliness, and development,

Haj is a migration to Allah and his prophet, (ﷺ), leaving everything - power, wealth, prosperity and leaving in complete humbleness and slavery, equal with your kin Muslim... as it is said; "No Arabic is better than a non-Arabic, nor a white is better than a black man except by piety", and these acts of worship protects and restrains man from evil doings, thus leaving them will lead to the spread of evil deeds and man will gain nothing but punishment for what he had done.

In order to complete the building of man and society, and to achieve the goals of Islamic Law, the doctrines of lawful and unlawful were put down to guide man to the right road and bestow happiness on him; as the in lawful deeds man will find his happiness, and in unlawful deeds he will be perished; thus the

prohibition of drinking alcoholic drinks, and all ways leading to it, as prescribed by Allah was for the protection of man's mind and body, the society from diseases and the consequences of the absence of his mind, the prohibition of adultery, and all ways leading to it, wanton display of beauty, solitude with a woman, and libertinism... etc, was prescribed to protect the family and the whole society from dissociation and mixing of lineage which destroy the society, thus the philosophy of prohibition in Islam is meant for the prevention of harms to man himself and to others as well.

Thus, it is clear that the goals of Islamic Law (*Sharia*) can not be achieved without good health and well being, as Abu-Al-Dardaa said to the prophet, (ﷺ), "To be healthy and grateful, is much more better than to be ill and endure patiently", the prophet (ﷺ) answered him by saying, *"Allah loves healthy people, as you do"*.

That is not all, but Islam's view of the sick and sickness has overrun all that preceded it and whatever followed from laws or social systems, as Islam does not see sickness as an anger of Allah, or a touch of the devil, but a trial, and the Muslim has to be patient and bear it with patience as the Prophet (ﷺ) said,

> *"Any kind of sadness or grief or even the prick of a thorn that inflicts man is a blessing from Allah as He raises him a degree higher or takes from his bad deeds instead".*

The Holy Quran came to the world with statements about the inner self, this was fourteen centuries ago, and it put to it four marvelous divisions in various parts of the Holy Quran, thus the world knew about the peaceful innersoul, the lamenting innersoul, and the authoritative innersoul. Abu-Hamid Al-Ghazally, has delved deep in the inner-self in his encyclopedia "The Revival of religious sciences", under the heading" Fear and Request", as the Holy Quran talked about the ailments of the heart, and their different kinds, as it was mentioned by Imam Al-Zahaby in his book "The Prophetic Medicine".

As for the medicine of the heart, it is only found in the sayings of the benevolent and kind Prophet (ﷺ), when he quoted Allah, the only source of all knowledge, he says that for the hearts to be righteous, it must know its creator, His names, characteristics, deeds, orders, and prohibitions and anger, as there is no way of being righteous except by doing this, and no way of getting these advice except from Mohammed (ﷺ).

Imam Ibn Kerium Al-Jozeiah has divided the hearts into two divisions: suspicion and doubt, and desire and error. He quoted the Holy Quran as saying,

"IN THEIR HEARTS IS A DISEASE; AND GOD HAS INCREASED THEIR DISEASE".

(Al-Baqarah: 10), and:

"O CONSORTS OF THE PROPHET! YE ARE NOT LIKE ANY OF THE OTHER WOMEN: IF YE DO FEAR (GOD), BE NOT TOO COMPLAISANT OF SPEECH, LEST ONE IN WHOSE HEART IS A DISEASE SHOULD BE MOVED WITH DESIRE"

(Al-Ahzaab: 32).

The Quran described the inner-self when horrified or frightened, and how to make it peaceful again in His very simple and clear words:

"TRULY MAN WAS CREATED VERY IMPATIENT; FRETFUL WHEN EVIL TOUCHES HIM; AND NIGGARDLY WHEN GOOD REACHES HIM; NOT SO THOSE DEVOTED TO PRAYER: THOSE WHO REMAIN STEADFAST TO THEIR PRAYER; AND THOSE WHOSE WEALTH IS A RECOGNIZED RIGHT FOR THE NEEDY WHO ASKS AND HIM WHO IS DEPRIVED (FOR SOME REASON FROM ASKING) AND THOSE WHO HOLD TO THE TRUTH OF THE DAY OF JUDGMENT; AND THOSE WHO FEAR THE DISPLEASURE OF THEIR LORD, FOR THEIR LORD'S DISPLEASURE IS THE OPPOSITE OF PEACE AND TRANQUILLITY."

[Al-Maarij: 19-28].

This is how Islam considers health, which was defined by the prince of Islamic physicians: Ibn-Sina by saying: "Medicine is the science by which the human body is known, and what is good and what is not for being healthy or otherwise." This comprehensive definition which was introduced more than one thousand years ago, is nowadays adopted by the WHO, that health is the state of the healthy body, mind and society, not only the lack of diseases or inability.

In spite of this definition of the WHO, during the forties, it ignored the spiritual side, which shows the lack of a comprehensive view of Islam about health, as Islam defines health from all domains, bodily, spiritually, psychologically and socially, and this last definition came 14 centuries ago, by the Muslim physicians.

To reach these noble goals, and great objectives for the Lord's heir on earth, there had to be a way to keep man healthy, and this is by the science of medication which was considered by the Muslim religious scientists an ordinance in the Islamic world, and Imam Al-Shafeiy said about it; "There is no knowledge, better than the prohibited, and non-prohibited acts, to my knowledge, except the science of medication". Dawood Al-Antaky in the introduction to his famous prescription says that there is no science that can do without the science of medication, because no acquisition of any knowledge is perfected without a sound body, senses, and mind.

Islam has taken good care of the different branches of medication; protective, preventive, an rehabilitative; in the protective, many sayings of the prophet (ﷺ), called for protection, in order to keep health in all its branches - cleanliness, food organization, and many healthy habits, as well, the researches in this domain is varied and all are derived from the prophet's (ﷺ) wise sayings, no need to repeat them here.

As for the treatment side Islam legalized medication, and the prophet (ﷺ) ordered medication and looking for it when he said:

"Ye believers, get treatment, the Lord created no disease without its medicine, known to those who know and ignorance to those who don't know".

As for rehabilitation, we are asked to look for it, he allowed one of his disciples to put a piece of gold on his lost nose during his invasions.

As for the three opinions pre-mentioned concerning the definition:-

To the first group we say: Medication is a human heritage and contribution, but the human thinking has deviated from the right path, and religion is in the church and in the mosque or the temple, due to their sufferings from the control of the church over medication and sciences, and making them only for the priests, medication did not develop, and the ship of science sank deep with its arsenal of destruction, thus they produced the microbial bombs, and medication turned into fatal poison; instead of relieving pains, and becoming a tool of the Lord's benevolence, it became devastatingly harmful, and the brother became keen on eliminating his human brother, and the call for killing substituted the call for mercy, the organs began to be sold, and man was transferred from the master of earth to a sample in labs, and source of trade .... etc. the list is endless.

The best evidence to be quoted here is the saying of Abenhaimar; the father of the atomic bomb, when he saw it explode in Hiroshima from a distance, he said his famous words: "Now, and now only, science has sinned".

As for the second group, which said: "Islamic medicine is nothing but an ancient memory and a call for underdevelopment.." we say to them that the heritage of any nation is like the roots of a tree, whenever it goes deeper and deeper in history, it becomes firmer and firmer and provides it with the means of living; the invention of genetic engineering, the nuclear bomb, and organ

transplantation are not only signs of civilization, but they are the leaves of the tree and its fruits, as civilization is much more wider than that, and cares less with its achievements, but cares more for the achiever, MAN, and cares for the philosophy of his existence in this world and the hereafter, as well as his ethics and culture.. if he is separated from these, he will be lost for ever. Now although the western man enjoys the highest per capita, and has got every means of prosperity, we find the percentage of suicide going up and up, as well as the addiction of narcotics, drugs... etc. became a daily practice; to enable him to forget and escape from his worries... the western man neglected the spiritual side of feeding his inner-self, and instead tried to feed on earth's food, thus he failed, and was transferred to a cog in a big machine.

This is not only in the west, but it is now prevalent in the east, as well; family relations are severed, social relations collapsed, man changed into a wild beast in a jungle full of fierce animals, each is trying to eat the other. I don't want to say more, it is enough to remind you with the AIDS that is harvesting man's bodies... Nevertheless, no body talks about chastity, virtue or ethics.. but they began to distribute contraceptives, for males and females, as if saying "Do it however, and whenever you want..! but use these contraceptives to protect you from the AIDS..!" Is this the Islamic way or attitude towards the man, whom it honored and asked to walk and learn and enjoy the fruits of life. Man asks, as many asked before about health and happiness, in spite of his materialistic progress and scientific development in all fields of medicine and protective treatments.

Islam gave due attention to man's environment, and warned him against corruption and doing mischief, as both affect his health, the Lord's words describe what happened all over world from corrupting the environment, which threatens man's life as He said' "CORRUPTION HAS APPEARED ON LAND AND IN

SEA ON THE HANDS OF MAN, TO MAKE HIM TASTE SOME OF HIS DOINGS, HOPING HE MIGHT RETURN TO RIGHTEOUSNESS", and He orders us not to do mischief by saying, "DON'T CORRUPT THE EARTH AFTER IT HAS BEEN RECLAIMED." Corruption here, I believe is both materialistic and ethical; as material corruption includes mischief on earth and around it, and ethical corruption means self and moral corruption.

To add to all these views that each civilization has its characteristics, its features, its morals, and its practices, Islam is unique in this, as Islam sees man as a whole, body and soul in full balance, none overweighs the other, as he did not worship the material, nor invented priesthood. Islam has taken care of man before he was born, when choosing a wife or a husband, at marriage, when he was a sperm drop, a baby, young, and old, Islam put to him a very accurate disciple system of life, taught him how to eat, drink, dress, treat himself, his Lord, his family, and his community. Islam has put to him goals in life - as it is a farm for the hereafter, to harvest from what his hands grew, and Islam was able to introduce a civilization to the world, with which Europe progressed from its dark ages with the help of the Islamic doctrines, but the Muslims slackened down and left Europe to lead the ship of scientific development. It may be that our interest in calling medicine by the Islamic Medicine, came as a symbol to awaken the Islamic world, and tell them that there is a lot in Islam in all fields; economics, architect, arts, cosmetic, medical... etc. and their commitment to Islam will bear fruits, too. One objective of choosing this name to medicine is the human deviations in practicing medicine in the West, but the East has to have a loud voice to awaken it and shake it; that is the voice of Islam, by providing the right opinion in these practices, especially when we lost the lead of materialistic science, but we can still provide it with

what purifies them and saves them from deviation, this is by means of the enlightened Islamic views. Moreover, the communication revolution has made the world a small village, knowing what happens all over it by the second... these developments are knocking our doors, thus we must be aware of it and give the Islamic view point in it, showing the advantage of Islam which differentiates between what is right from what is not.

The Lord knows what the inner-self whispers, as He is nearer to him than his vein, and He is the maker of his inner-self, and He directed him to his success, as He says'

"BY THE SOUL, AND THE PROPORTION AND ORDER GIVEN TO IT. AND ITS ENLIGHTENMENT AS TO ITS WRONG AND ITS RIGHT. TRULY HE SUCCEEDS THAT PURIFIES IT, AND HE FAILS THAT CORRUPTS IT".

(Al-Shams: 7).

The Almighty knows what the corrupt eye sees and what is hidden in the hearts.

Some people suggested that we call it THE ARABIC MEDICINE, in order not to distort the picture of Islam, as a result of misdemeanor of some practitioners, but this name might lead to the understanding of the use of medicinal plants and ancient medication practices, and this has its shortages, as well as its advantages, too, and because most of those who enriched the Islamic movement were not from the Arabic environment, like Al-Razy - from Al-Rey, Ibn-Sina - from Russia, and Al-Bukhary - from Tashkand... etc and thus we'll enter into the vertigo of apartheid, but Islam had engulfed them all. Moreover, if we want to discuss the point of view of Islam in modern things, on what ground shall we argue? Are there Arabic foundations? or, all the foundations taken from the Islamic Law (*Shareeaa*)? Thus the best name was "THE ISLAMIC MEDICINE", which is nearer to the fact, as for the fear of the misbehaviors, which might be alluded to Islam, wrongly, we know

that all Adam's sons are sinners, and the best sinners are the repentants, we are in a stage trying to erase eras of Islamic decay and weakness, we want to contribute to Islam and to be affiliated to it again, as well as to revive its name and face all over the world, and to prove that its doctrines are applicable, and their consequences are guarantee for man's well being and prosperity.

The Organization aims, also, at retrieving the Islamic behavior which was defined to Man by Islam, and make part and parcel of his daily conduct; if cleanliness, for example, is part of the belief, as said by the prophet (ﷺ), we find our Islamic states are the least countries enjoying and abiding by this Islamic ordinance, although it is the main road to health, and there are many wise sayings which organize the life of the Christians as well as the Muslims in order to lead a healthy and clean life, in the same way the orders and prescriptions in Islam are all related to man's psychological, social and body health; like prayer, fasting, *Zakat, Haj*, and others of the ordinances that have spiritual meanings which invests in Man tranquility and protects him form psychological and body diseases. There are many researches reinforcing these hypotheses, and the things that Islam forbids us from doing are essentially for our sake, we are not far away from what the world is suffering from narcotics, alcoholic drinks and AIDS which Islam prohibited.

We also wanted to utilize the plants which we have as a gift from the Lord, and Muslims have surpassed the world in this field, thus they kept their heritage of plants for the future generations, moreover they added and developed it. They wrote many books from which the Europeans took and translated and utilized till the 19th century; all their experiments and observations built on high scientific standards: Al-Hawy is considered the first scientific clinical encyclopedia in the history of the medical sciences.

Islamic civilization, at that time, was able to open its arms welcoming every active worker, Muslim or non-Muslim, as Islam

has no discrimination, and no coercion in religion, no one is better than the other except by worship and good deeds, thus scientists migrated to it from east and west to add to its sciences.

I'll mention here, only, the testimonies of some Western scientists for the Islamic civilization:- "Froje Garoody" talks with sadness and grief about western Civilization; he said. "The Western civilization is dying and committing suicide because it deviated from following the natural disposition; the instinct, and its masters considered man the director of the nature which he ruled, but after five centuries of the experience we found out that Nature is the main store of the primary materials and the place for man's leftovers, this made us always destroy nature, and this is against what the Holy Quran decided, as it decided that man is the Lord's heir on earth, and man is concerned with keeping natural balance"; then he says; "Our present western civilization is dying, not because it is short of means, but because it lacks goals". Man began to threaten himself with annihilation, and the result is the destructive weapons that man possesses are enough to destroy the planet earth one hundred times, what poor creatures we are!

This civilization is carrying in its womb the causes of its destruction, on the contrary of the Islamic civilization because the Islamic civilization is coming from the Lord who made it, not man, nor is the Islamic civilization an extension of history, but a revelation from the Lord to His prophet (ﷺ) through the Holy Quran, dictating a Holy Constitution satisfying the body and the spiritual needs of the human beings, then following this came the wise sayings of the prophet (ﷺ) to explain the quranic doctrine, thus everything became clear, the lawful is clear and the unlawful is clear, and the difference between them is clear. The world is about to face a crisis due to its losses from addictions, as the costs of these addictions reached 14 billion $ in one year in the USA only, and these losses were in work hours, accidents, family problems... etc.

due to the addiction of narcotics or alcoholic drinks, which Islam prohibited. This big sum of lost money is more than the revenue of many countries, and the world will face more than 40 million individuals inflicted with AIDS by the year 2000, and 10 million orphans; the WHO estimates the number will be doubled, nevertheless, virtue is absent, chastity killed, and they don't know where they are going... and no body knows!

Max Mayerhoof testifies: "The Islamic medicine has reflected the sun shine which was setting in Greece, and the moon glittered in the sky of the dark ages, and other stars brightened by themselves and lit the gloomy dark sky, then the moon went down and the light of the stars waned in the revival age, but their traces are still there, to be felt in the civilization of today.."

Montgomry Watt said; "I'm not going to look at Muslims as a barbaric army invading Europe, but I'll consider them the representatives of a civilization which achieved great successes all over the world, spread them to their neighbors. The Europeans are not appreciating their debt to the Islamic Civilization!! They even try to find faults with the volume of the Islamic effect and its importance in our cultural heritage, forgetting, again, that our good relations with the Arabs and the other Islamic nations calls upon us to be aware, to the end, that we owe them, not to mention this truth, or its denial is not right..."

Montgomry Watt didn't stop at that, but he added, "Our following the Arabic Medicine, which lasted till the 15th and the 16th centuries is evidently clear in the printed books, and the first of these books was explanations of the 9th chapter of the Principles of Al-Razy, then followed the printing of Ibn-Sina for three times, before Galinos, and till the year 1500 sixteen editions of "Al-Kanoon", the "Law". The statistics show that the quotations and extracts found in the early European writings are evidence that the

impact of the Arabic books surpassed and surmounted the Greek one.

He says, too, "Islam in essence is not only a mere religious movement, but it is also a human value embedded in life of the peoples who embraced Islam, or joined it, it was a kind of unique human existence in the world as the conditions of the Islamic openings were to permit the other people to continue practicing their former habits, laws, and languages, for paying taxes (*Jiziah*), these Islamic rules strengthened the relations between the Muslims and the peoples of the countries they conquered, thus the people continued to practice sciences, arts and especially medication.

These three testimonies are only a sample, there are a lot of others for which there is no space to quote here, but in time we will.

In addition to this, the last WHO statistics mention that 25-30% of the diseases from which man suffers nowadays are caused by the side effects of the chemical medicines, as well as their high prices, and the expertise which they need to manufacture. Contrarily, however, our Islamic countries enjoy a suitable weather for the medicinal plants to grow and treat a lot of diseases. All we need are issuing political decrees as China and India and other nations which produce these medications in the most modern fashion.

This is a short synopsis about the idea of Islamic Medicine, and to reinforce this idea, we invited a group of Muslim thinkers to take part in many conferences to write in this field, and we have received a lot of their contributions which will be published in due course of time, under different headings.

SUMMARY OF THE RESEARCHES IN THIS BOOK

This book embodies 25 research publications on the pharmacological, and clinical studies on several medicinal plants, namely - *Nigella sativa, Berberis aristata, Terfeziz claveyi, Butea monosperma, Pistacia lentiscus, Aloe vera, Myrtus communis, Glycyrrhiza glabra, Cassia* species, *Catha edulis, Paeonia emodi, Poterium spinosum, Allium sativusm, Emblica officinalis, Achyranthus aspera, Salvadora persica, Alhagi mannifera, Astragalus spinosus, Sphaeranthus hirtus* and *Euphorbiaceae* species, commonly used in the Islamic Medicine.

The first three papers are devoted to *Nigella sativa.* This plant has been found to enhance the immune functions in human voluteers, as manifested by improved helper suppressor T cell ratio and an improved natural killer cell function activity (El-Kadi and Kandil). The carbonyl fraction of the essential oil of this plant possessed uricosuric, choleretic, and protective activity against histamine-induced bronchospasm (El-Dakhakhany) and its extracts inhibited the fibrinolytic activity and shortened the bleeding time (Ghoneim, *et al*).

The next two papers describe the experimental studies on *Berberis aristata.* Its crude extract as also the active principle, berberine, have been reported to singnificantly reduce the incidence and severity of diarrhoea induced by cholera toxin and by other diarrhoea-provoking agents (Sabir, *et al*). Berberine also exerted antiheparin and antitrachoma actions in that it specifically neutralized the *in vitro* anticoagulant action of heparin, and inhibited the development of elementary bodies on the yolk sac membrane and protected the chick embryos from the lethal effect of trachoma organisms and also controlled the experimentally-induced trachoma in the monkey eyes (Sabir). Incidentally, another plant, *Trafeziz claveyi* (Truffles) has been found clinically useful in

trachoma in human subjects wherein it hindered the process of fibrosis, interfered with fibrocyte formation, prevented the abnormal growth of conjunctival cells and promoted vascularisation (Al-Marzooky).

Butea monospera flower has been found to offer protection against carbontetrachloride induced hepatotoxicty in albino rats, as was assessed by biochemical and histopathological studies as also by the pentobarbitone-induced sleep and by the rate of liver regeneration in partially hepatectomized rats (Nazimuddin, *et al*). Further, it exerted sigificant antiinflammatory effects in rats and its effect was comparable to that of phenylbutazone (Nazimuddin and Khaleefatullah).

The clinical trial of *Pistacia lentiscus* (Mastic) revealed that in a single daily dose of 1 gram for two weeks it produced complete symptomatic relief in 80% and endoscopic healing in 70% patients suffering from duodenal ulcers, and this effect was statistically significant over the placebo (Al-Habbal *el al*). This plant was found equally effective in the treatment of benign gastric ulcers (Al-Habbal and Huwez). On experimental evaluation, another plan *Aloe vera* has been found to protect gastric mucosa against lesions produced by chemical and nervous stress in rats (Kandil and Gobran).

Amongst several plants screened for their antibacterial activity, *Myrtus communis* was found to be most effective although some others also possessed varying degree of antibacterial efficacy. Being encouraged with the antibacterial activity of this plant against *E. coli* and *Shigella dysenteriae* an antidiarrhoeal oral emulsion and a cream for topical application has been prepared for clinical trial (Haq). In another study (Ozkal *el al*), four species of *Glycyrrhiza glabra* have been screened for their antimicrobial activity and all were found effective against *Staphylococcus aureus* and *Mycobacterium tuberculosis*. Notably, plants do posses antiviral activity as

well, for the antiviral screening of eight species of genus *Cassia* revealed that *C. siamea* had the maximum inhibiting capaity for TMV while others had little or no effect (Misra and Sinha).

Psychopharmacological effects and the status of uses and abuses of *Catha edulis* (Khat) have been reviewed by Ageel *et al.* The active principles of this plant, cathine and cathinone, have been reported to possess amphetamine like actions on gross behaviour, body temperature, locomotion, stereotyped and operant behaviour and food intake in experimental animals. They also enhance electrically stimulated noradrenergic transmission, the mechanism of action is believed to be the release of neurotransmitter at the end of noradrenergic neurons. On the otherhand, *Paeonia emodi* has been reported to produce marked CNS depression, and anticonvulsant effect against supramaximal electroshock and pentylene tetrazole induced convulsions in rats (Ahmed, *et al*).

In Jordan, *Poterium spinosum* is reputed for its antidiabetic properties. Experimental investigation of this plant has vindicated its use by the common people, as it was found to be effective in alloxan-induced diabetes in rats (Waheed and Rahman). Likewise, the hypocholesterolemic effect of *Allium sativum* has been confrmed experimentally and its potential protective action against coronary heart disease discussed (Ahmad). General pharmacodynamic studies on *Emblica officinalis* revealed that this plant possesses powerful expectorant activity in addition to having antibacterial action; on cardiovascular system it acts like adrenaline and ephedrine (Siddiqui). And, *Achyrenthes aspera* has been found to reduce the gastric acid secretion in rabbits (Qadry, *et al*).

Clinical evaluation of *Siwak (Salvadora persica)* in human subjects indicated that it could act as an effective device in preventive dental care programmes in mass population, as was seen by its effect on the plaque formation percentage and gingivitis percentage (El-Mostehy, *et al*).

Preliminary pharmacological investigations on *Alhagi mannifera, Astragalus spinosus* and *Sphaeranthus hirtus* has been carried out by Ghoneim *et al.* The former two plants were found to posses antispasmodic and smooth muscle relaxant properties besides exerting antihistamine and antiserotonin actions, and the third plant, in addition, also possessed bronchodilator and coronary vosodilator effects.

In the last paper, irritant and co-carcinogenic properties of some plants belonging to *Euphorbiaceae* family has been demonstrated, and the use of *Croton tiglium* and *Euphorbia resinifera* has been recommended to be banned (Miana). However, further systematic studies are required to be undertaken before such a step is taken.

EFFECT OF NIGELLA SATIVA (THE BLACK SEED) ON IMMUNITY

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U.S.A.

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Our interest in *Nigella sativa* (the Black Seed) was initiated by the authentic saying of Prophet Muhammad (ﷺ):

"In the Black seed there is healing for every illness except death"[1] by Al-Bukhari

Nigella sativa belongs to the family of Ranunculaceae. Its seed also known as black seed, black caraway, black cumin, and by several other names, has been in use in many Middle Eastern and Far Eastern countries as a natural remedy for over 2000 years. In Egypt it is called *"Habbat-ul-Barakah"* which means the seed of blessing.

The seed is black and minute possessing an aromatic odor and taste and is frequently added to bread as a flavoring agent. As a natural remedy people take it minced by itself or mixed with honey, or use its oil, either as a promoter of good health or for the treatment of a variety of ailments.

In Unani Medicine it is used as detergent, digestive, carminative, stomachic, laxative, antibilious ,anthelmintic, diuretic, lithontriptic, emmenagogue, galactogogue, stimulant, antiphlegmatic, expectorant and local anesthetic. Dawood Al-Antaki (in his famous ancient prescription book) identified the seed and used its oil in the treatment of bronchial diseases[2]. Mahfouz and El-Dakhakhny in 1959 isolated the cyrstaline active principle "Nigellone" with the chemical formula $C_{18}H_{22}O_4$ from the oil of *Nigella sativa* seeds[3].

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They documented the ability of Nigellone to prevent histamine-induced bronchospasm in the experimental animal.[4] Clinical studies have also shown a beneficial effect of nigellone in the treatment of patients with bronchial asthma.[4] In the experimental animals,[4] Nigellone was found to be free of any irritant or toxic effects whatsoever, even when injected in large doses[2]. Other studies showed that the extracts of *Nigella sativa* seeds have a marked choleretic effect causing an increase of bile flow,[5] an antibacteral effect,[6] and hypotensive effect.[7]

In view of the multitude of uses of *Nigella sativa* where its effectiveness was documented, and primarily because of the above listed Prophet's (ﷺ) saying indicating that there is healing for every illness in the Black Seed, we suspected that the Black Seed may have some stimulating effect on the immune system of the human body. The purpose of this study is to evaluate such an effect if any is present.

What is the immune system?

We may compare the immune system to an army and a police force which protects the body against invading harmful matters, micro-organisms, and cancer cells. It is the body's own defence against infections and cancers. The job is carried out, by specialized cells such as the various types of T-lymphocytes, B-lymphocytes, and Macro-phages; and several bio-chemical compounds - proteins - produced by these specialized cells. The T cells are the lymphocytes which has special training in the thymus gland. There are three specialities among the T cells: The helper T cells (or T_4) which activate the immune battle and give instructions to other cells telling them what to do: the killer T cells (or Natural Killer cell) which kill the invading enemy units; and the suppressor T cell (or T_8) which give signal to the other cells to end the battle. In normal health the number of the helper T cells is twice the number of the

suppressor T cells. The bio-chemical compounds include Interleukin 1 and 2 (IL-1, IL-2), Tumor Necrosis Factor (TNF), Gamma Interferon (IF), B-Cell Growth Factor (BCGF), B Cell Differential Factor (BCDF), and a huge number of Antibodies. The battle starts when the macro-phages (the big eaters) meet the enemy units, be it a virus, a micro-organism, or a cancer cell. The macro-phages consume some of the energy units, seize their antigens and display them on their own surfaces. Among millions of helper T cells circulating in the blood stream, a select few are programmed to read that antigen. These helper T cells couple with the marco-phages. We may call this the vital union which starts the activation process of the T cells. The macro-phages secrete the lymphokine Interlenkin 1 (IL-1) which activates the Helper T cell. IL-1 also stimulates the brain to raise the body temperature causing fever which enhances the activity of the immune cells. The activated helper T cell produces another lymphokine, Interleukin 2 (IL-2) which activates other helper and killer T cells to grow and multiply. The activated helper T cells also produce the lymphokine B Cell Growth Factor (BCGF) which causes the B Cells to multiply. As the number of B cells increases the helper T cells produce another lymphokine, the B Cell Differention Factor (BCDF) which instructs some of the B cells to stop replicating and start producing antibodies. The helper T cells also produce a lymphokine called Gamma Interferon (IF) which has multiple effects. Like IL-2 it helps activate Killer T cells enabling them to attack the invading enemy units. Like BCDF, Gamma Interferon increases the ability of B cells to produce antibodies. It also affects macrophages, keeping them at the site of the battle and helping them digest the cells they have consumed.

Then the Killer T cells, which were recruited and activated by the Helper T cells, start performing their specialized job which is killing cells of the body that have been invaded by foreign organisms, as well as cells that have become concerous. The

antibodies which were produced by the B cells rush to the battle field where they either neutralize the enemy units or tag them for attack by other cells or chemicals.

When the enemy units are conquered and controlled, the Suppressor T cells halt the entire range of immune responses preventing them from going out of control. They slow down or stop the activities of the B cells and other T cells, Memory T and B cells which are defense cells generated during the initial phase of the battle, are left in the blood and lymphatic system where they will circulate for years to enable the body to respond more quickly to subsequent attacks by the same enemy.

Studies to evaluate the immune system

Among numerous studies available to evaluate the immune system we currently perform the following studies and feel that they offer adequate indication of the effectiveness of the immune system of a given person.

The total count of B cells and T cells, the count of the sub groups of Helper T cells (T4) and Suppressor T cells (T8), the ratio between T4 and T8, the Natural Killer cell activity, and the various Immune Globulins representing the antibodies. We may add additional functional studies to our immune profile testing in the future.

For better understanding of the results of the Natural Killer cell activity studies it should be explained that the test is done by incubating isolated Effector cells, which are the Natural Killer cells of the person to be tested, with Target cells, which are cancer cells grown in the laboratory in a tissue culture medium. The Target cells are labelled with radio-active chromium 51 (^{51}Cr) prior to the incubation with the Effector cells. The mixing of Effector and Target cells is done in 3 different dilutions, or Effector: Target ratio (ET ratio) of 10:1, 50:1, and 100:1. The degree of cytotoxic activity

of the Natural Killer cells, which corresponds to the degree of lysis (or death) of the cancer cells is determined by measuring the amount of released radio-active material in the supernatant fluid with a Gamma Counter. A different activity level is measured for each of the three dilutions, or Effectors to Target ratios.

METHODS AND MATERIAL

Apparently healthy volunteers were randomized into 2 groups. One group received one gram of ground *Nigella sativa* seeds twice a day, while the other group received activated charcoal powder as a placebo. The ground *Nigella* seeds as well as the placebo were packed in identical capsules. The identity of the capsules was known only to one of the investigators. The volunteers did not know which kind they were taking nor did the other investigator who was incharge of performing and interpreting the immune studies. The code was not broken until all the results were available for every volunteer. The results which are presented in this report are those of 27 volunteers, 16 males and 11 females, ranging in age from 10 to 60 years, with an average age of 33 years. Among the 27 volunteers there were 22 Muslims and 5 Non-Muslims. Eleven of the 27 took real *Nigella*, 12 took Placebo and 4 volunteers took Nothing. Two of the four who took Nothing were supposed be in the *Nigella* group, while the other two were supposed to be in the Placebo group. These four did not take their capsules for various personal reasons. The groups of *Nigella* and Placebo were reasonably well matched.

The initial protocol had one hundred volunteers on it for the study. The results which were indicated in the earlier abstract were the preliminary results of the first 10 volunteers. For some technical and financial reasons the studies could not be completed in time for this presentation except for the 27 volunteers which are the subject of this report. All volunteers had their immune profile evaluated

before the study and 6 weeks after the study was started. The immune profile included a complete B cell and T cell count including the T cell subgroups of Helper T cells (T4) and Suppressor T cells (T8). It also included the Natural Killer (NK) cell functional activity assay, and the measurement of the Immune Globulins IgA, IgG, and IgM. The two main indicators for the effectiveness of the immune system would be the T4:T8 ratio, and the NK cell functional activity level. An increase or enhancement of either one is considered an improvement while a reduction or decline is considered a deterioration.

RESULTS

In the *Nigella* group there were varying degrees of enhancement of both the T4:T8 ratio as well as the NK cell activity level in the majority of subjects while a few had a decline. The net results indicated an improvement of the T4:T8 ratio from 1.19 to 1.85 which is a 55% improvement. The net enhancement of NK cell activity was 24% at 10:1 ET ratio, 10% at 50:1 ET ratio and 42% at 100:1 ET ratio. The immune globulin levels had an average decrease of 32.5% for I_gA, 12.6% for I_gG, and 29.4% for I_gM.

In the activated charcoal group the T4:T8 ratio showed an average decrease from 1.27 to 0.95, which is a deterioration of 25%. However, all subjects taking the charcoal showed varying degrees of NK cell activity with an average improvement of 63% at 10:1 ET ratio, 56% at 50:1 ET ratio, and 66% at 100:1 ET ratio. The immune globulins showed an average decrease of 27.4% for I_gA, 19.4% for I_gG and a minimal increase of 1.4% for I_gM.

In the third group who took Nothing there was an average decrease of T4:T8 ratio from 1.1 to 0.97 which is a 12% deterioration. There was also a decline in the NK cell activity at all dilution levels in all subjects averaging 32% for 10:1 ET ratio, 23% for 50:1 ET ratio, and 19% for 100:1 ET ratio. The immune

globulins in this group showed an average decrease of 8.9% for I_gA, and 3.8% for I_gG, but an average increase of 90% for I_gM.

Reported side effects included one volunteer in the *Nigella* group reporting that he was not losing his hair as he used to. Another one in the same group reported recurrent indigestion necessitating discontinuation of the *Nigella*. In the charcoal group one reported that her finger nails became much stronger. No other significant side effects were reported.

DISCUSSION

In addition to the confirmation of a positive stimulating effect of *Nigella sativa* (the Black Seed) on the immune system, the results of this study gave us several surprising but very useful pieces of information. The biggest surprise was the significant and real enhancement found in the charcoal group. A positive Placebo effect might be expected in up to 30% of subjects, and to a degree of improvement of up to 30%. In this group all the subjects had improvement, and to a level much higher than would be expected from a Placebo. Even more impressive was the fact that all of the subjects who took Nothing had a significant decline. This shows two things: First that activated charcoal was the wrong choice as a Placebo. Second, and this was the very pleasant surprise, that charcoal caused a significant improvement of the immune functions. The puzzling question remained, how did it do it? Charcoal is not supposed to be absorbed from the intestinal tract, and therefore it was not expected to have any direct effect on the immune system. This continues to be true. The effect is apparently an indirect one through a different mechanism which we did not consider initially. Activated charcoal has the ability to absorb toxic chemicals in the digestive tract and is often given by mouth as a treatment following ingestion of toxic material. It is frequently used in water filters to absorb chlorine and other chemical impurities in the water.

Apparently what happened in our charcoal group is that the charcoal absorbed all toxic chemicals contained in the ingested food and drinks, and left only the clean pure natural nutrients to be absorbed through the intestinal tract. But were there any toxic chemicals in the food and drinks ingested by these volunteers? Apparently, Yes! Nutritionists and members of the so called "Health Movement" have been claiming for years that all the artificial chemicals added to the food and drinks are harmful and may be responsible for suppressing our immune system leading to increase of cancers, infections, and other diseases; and should therefore be avoided. These artificial chemicals are in the form of a huge number of preservatives, artificial colours, artificial flavours, bleaching agents, many food additives, industrial pollutants to water, air, and soil which may again affect drinking water or plants growing from contaminated soil, and the list goes on and on. Once these harmful toxic chemicals were eliminated from the food and drinks by means of the charcoal, their suppressive effect was lifted away from the immune system, and the immune cells were allowed to grow and flourish without restriction. These facts about pure natural nutrients and their effects on the immune system have been known for years but were mainly based on logic and indirect evidence. To my knowledge, this is the first time that these facts are confirmed in such a direct and clear way as it happened accidentally in our study.

Another interesting finding was the fact that these so called "healthy volunteers" were not really that healthy. The majority of them had a somewhat impaired immune function. We tried to see if those with impaired immunity had something in common and indeed they did. The majority of these volunteers worked at or belonged to families of those who worked at the Akbar clinic and its affiliated projects. All of these are under a considerable degree of stress, mostly financial, and otherwise too. In the *Nigella* group we

found that the few who had a decline of the NK cell functions had a particularly high level of stress during the period of the study due to sickness in the family or some other hardship, making their share of stress higher than the average member of the group. The stress situation to which most of the participants of the study were subjected would also explain the fact that all volunteers who took Nothing had deterioration of their immune functions. Of course, there is nothing new in the fact that stress impairs the immune functions. This has been proven in many other studies. The significance of this finding in our study is that the actual real improvement caused by the *Nigella* or the charcoal is higher than the given figures. Because, if it were not for the stimulating agent (be it the *Nigella* as the pure natural nutrients), the decline would not be only down to the Zero level, but it would have gone lower to the level of those who took Nothing. Therefore, one could probably add the decline figures in the "Nothing" group (i.e. 32%, 23%, and 19%) to the enhancement figures in the other groups. This would make the real net enhancement figures for *Nigella* close to 56%, 33%, and 61% in the various dilutions.

Other factors to be considered while reviewing these results are: First is the fact that we have tested only one dosage of *Nigella*, i.e. one gram twice a day. A higher dosage may have a more potent effect. This has to be determined in future studies. Second is the fact that these volunteers, although under stress, were still relatively healthy and free of any cancer or other immune deficiency disorders. The improvement in such persons is expected to be limited anyway since they were not that far from normal to start with. If *Nigella* or the pure nutrients were tested in subjects who are really bad, the improvement would possibly be more dramatic. In our advanced cancer patients receiving our multimodality immunotherapy program (in which *Nigella* is one of several components) we see enhancements of NK cell activity not in the range of 50-60%

but in the range of 200-300%. We plan to have some controlled studies on isolated *Nigella* in some of these patients in the near future, *insha'allah*.

CONCLUSIONS

1. *Nigella sativa seeds* (the Black Seed) taken by mouth in a dosage of one gram twice a day have an enhancing effect on the immune functions, manifested in improved helper suppressor T cell ratio, and an improved natural killer cell functional activity.
2. The use of activated charcoal by mouth resulted in improved natural killer cell functional activity, most likely by eliminating the toxic chemicals from the ingested food and drinks, and allowing only the pure natural nutrients to be absorbed through the intestinal tract. The pure natural nutrients were thus able to exert their beneficial and immune enhancing effect.
3. Stress has a definite immune suppressive effect.
4. Additional studies are needed to confirm the above listed effect of activated charcoal, and to determine the effect of *Nigella sativa* on the immune system when given in different dosage and when used in subjects with severely suppressed immune functions.

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SOME PHARMACOLOGICAL PROPERTIES OF SOME CONSTITUENTS OF NIGELLA SATIVA SEEDS: THE CARBONYL FRACTION OF THE ESSENTIAL OIL

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SOME PHARMACOLOGICAL PROPERTIES OF SOME CONSTITUENTS OF NIGELLA SATIVA SEEDS: THE CARBONYL FRACTION OF THE ESSENTIAL OIL*

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The carbonyl fraction of the essential oil of *Nigella sativa* L. seeds was shown to be effective in protecting guinea pigs against histamine induced bronchospasm. It possessed very low toxicity; there was no effect on heart or blood pressure. It diminished the effect of histamine on the isolated bronchial muscle and ileum of the guinea pig. It also counteracted the stimulatory effect of adrenaline on the isolated rabbit uterus. This fraction possessed also a uricosuric activity when tried in rats. It also possessed a choleretic activity increasing the flow of bile with an increase in total solids in dogs.

Nigella sativa L. is an annual of the Ranunculaceae herbaceous plant growing in countries bordering on the Mediterranean Sea. The seeds and oil have been used since centuries for the treatment of different diseases by most of the common people. It has been reported that Prophet Mohamed (ﷺ) said:

"The black seed is a remedy for every disease except death".

It has been also reported in David's Prescription that the seeds can cure some chest diseases, cough, respiratory oppression, nausea and ascites and can be used as a diuretic, as an anthelmintic and as a preservative for food, and for many other purposes. These uses were later assured by some European authors e.g. Mattiolus and Bock[1].

Mahfouz and El-Dakhakhany[2] isolated a crystalline active principle from the essential oil of *Nigella Sativa* L. which was

* Bulletin of Islamic Medicine, 2: 508-511, 1982.

proved later to be the dimer of thymoquinone[3]. El-Dakhakhany found later[4] that the carbonyl fraction isolated from the volatile oil was polythymoquinone and it possessed lower toxicity than thymoquinone itself. So it was thought to study some of the pharmacological actions of polythymoquinone i.e. toxicity, uricosuric activity, choleretic activity and protective action against histamine - induced bronchospasm in order to reach a drug with low toxicity.

EXPERIMENTAL

The pharmacological investigations carried out were: toxic tests, uricosuric activity, choleretic activity, protective action against histamine-induced bronchospasm and anti-inflammatory effect.

The carbonyl fraction (polythymoquinone) used was the one isolated from the carboxylic acid fraction of the volatile oil of *Nigella sativa* L. seeds by Girard reagent[2]. Thymoquinone itself-used sometimes for comparison was isolated from the essential oil of *Nigella sativa* L. seeds by the use of silica gel column chromatography[3].

1. *Toxicity tests:* Male rats, weighing from 250 to 300 gm were used. The carbonyl fraction and thymoquinone were injected intraperitoneally dissolved in propylene glycol in doses varying from 5 mg up to 160 mg/kg body weight. The mortality rate of the rats was recorded against a control group which received only same amount of propylene glycol and was kept under the same condition. The LD50 was then obtained using the method described by Gaddum[5].
2. *Uricosuric activity:* This was studied in male rats, 250-300 gm in weight. Every two rats was placed in a metabolic cage. Food given was milk and bread. The urine was collected every 24 hours and uric acid was determined colorimetrically by the method of Benedict and Frank[6]. The uric acid in urine was

recorded as mg uric acid per day per cage. After establishing an average starting level for uric acid excretion over a period of 12 days, the drug under investigation was intramuscularly injected (4mg/kg body weight in propylene glycol) for successive five days. Each drug was given to 20 rats. Control experiments were also carried out where the animals were only given 0.2ml of propylene glycol.

3. *Choleretic activity:* Dogs weighing 10-14 kg were used. After initial anaesthesia with ether, barbitone sodium was given (0.22 gm/kg body weight) intravenously through the femoral vein. An upper median abdominal incision was made and the liver, gall bladder, common bile duct, cystic duct and hepatic duct were exposed. The cystic duct was tied to exclude completely the gall bladder. The common bile duct was then ligated just before entering the duodenum and L cannula was introduced so as to collect all the bile coming from the liver, through the hepatic duct. Bile was collected in special containers every 30 minutes, the volume was recorded and total solids were determined.

   Bile was collected at first during a period of 2-3 hours which represented normal secretion. The carbonyl fraction or thymoquinone was then slowly administered intravenously followed by a small volume of warm saline (about 5ml). Control experiments were carried out, only propylene glycol was injected. For each drug about 10 dogs were used.

4. *Protective action against histamine - induced bronchospasm:* Guinea pigs (200-250 grams) were chosen to be of the same sex and same body weight. The carbonyl fraction and thymoquinone were intraperitoneally injected (in propylene glycol) in different groups of animals. After about 2 hours, the animals together with controls (receiving only propylene glycol) were placed in a glass container and exposed to histamine mist (0.25% solution of histamine acid phosphate sprayed under a pressure of 0.5 kg/sq.cm). The time

elapsing before the onset of dyspneic conulsions was taken as a measure for the degree of protection imparted by the drug administered. When 10 minutes passed without the animal showing obvious signs, this was considered as complete protection.

5. *Anti-inflammatory effect:* This was carried out according to Selye[7]. To each group of 10 rats (about 200 grams weight) a cotton pellet was inserted under the skin to act as a foreign body. A daily dose of the carbonyl fraction and thymoquinone of 5mg/kg body weight was administered intramuscularly for seven days. Control experiments were carried out where the rats received only propylene glycol and in another group the rats received a daily dose of a standard anti-inflammatory agent namely 5mg/kg body weight of prednisone. The granuloma formation was determined in all the rats by weighing the cotton pellet.

RESULTS AND DISCUSSION

The toxicity tests showed that LD50 of the carbonyl fraction is 150mg/kg body weight and is far less than that of thymoquinone itself which is 10 mg/kg body weight.

The carbonyl fraction was found to possess a favourable uricosuric activity (Table I). In some rats, the uric acid excretion was doubled during the first day of treatment followed by a steep decrease which dropped sometimes below normal. The maximum uric acid excretion brought about by the carbonyl fraction was on the third day of treatment (Table II). The drug was only injected for five days, as in previous experiments[4], it was shown that the uric acid excretion dropped below normal despite administration of the drug. This can be accounted for by the limited stores of uric acid in rats. However, the uric acid level in urine started to return gradually to normal levels after stopping the drug during few days. The uricosuric activity of the carbonyl fraction was almost the same as

that of thymoquinone (Table I, II), but the carbonyl fraction, far less toxic, may favour its use as a uricosuric drug.

The carbonyl fraction and thymoquinone possessed also a choleretic activity, but thymoquinone was found to be more active in this respect. Both of them caused an increase in volume and total solid of the bile (Table III). It was found that the increase in volume of bile persisted for several hours while that of total solids was only for 1-2 hours and was maximal during the first 30 minutes after i.v. injection of both drugs. In the control experiments, it was observed that the collected bile was gradually less in volume and total solids. The total solids were approximately halved after about 6 hours. It was also noted, that the increase in volume and total solids after administration of the carbonyl fraction or thymoquinone was more obvious in dogs with low control bile volume and total solids i.e. in cases of hypofunction. In comparison to sodium taurocholate, it was previously found[8] that its effect started immediately after i.v. injection, reached maximum secretion in about 15 minutes and continued for a period of less than one hour.

The carbonyl fraction and thymoquinone protected guinea-pigs against histamine induced bronchospasm (Table IV), yet a higher dose of thymoquinone was required to impart this protective action. Consequently, all the guinea-pigs receiving thymoquinone died few hours after the experiment. The mechanism of this action is not yet fully clear although a direct action on the bronchial muscle has been previously proposed[9]. Mahfouz *et al*[10] observed an increase in the histaminopexic power in sera of asthmatic patients after treatment with the carbonyl fraction. An anti-inflammatory effect was not possessed by both drugs as the "granuloma-pouch" experiments showed that the drugs did not diminish the weight of the pellet than those of the control group.

Although the carbonyl fraction (polythymoquinone) and thymoquinone are close in structure, yet they differed to some

extent in their pharmacological properties; thymoquinone being sometimes more active but always more toxic. Polymerisation of thymoquinone with subsequent separation of the carbonyl fraction lowered to a great extent the toxicity of thymoquinone without appreciable loss of activity.

The carbonyl fraction may prove to be of favourable therapeutic value in cases of hepatic insufficiency, gout and in some cases of bronchial asthma. Preliminary reports are encouraging assuring the use of the carbonyl fraction for the above-mentioned conditions; it was used in very low dosage i.e. 6-10 mcg/kg body weight daily without showing any side effects even for long-term therapy.

SUMMARY

The pharmacological properties of the carbonyl fraction (polythymoquinone) isolated from the volatile oil of *Nigella sativa* L. seeds were studied. It was found that it possessed a uricosuric, choleretic and protective activity against histamine induced bronchospasm. On the other hand, it was devoid from any anti-inflammatory activity and possessed very low toxicity than the parent substance thymoquinone. This supports its use for the many purposes, the *Nigella sativa* L. seeds are reputed for by the common people, preferably without the other ingredients.

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TABLE I

URIC ACID EXCRETION DAILY BEFORE AND THREE DAYS AFTER INTRAMUSCULAR ADMINISTRATION OF THE DRUG (4mg/kg) TWENTY RATS IN EACH GROUP

DRUG	MEAN URIC ACID EXCRETED ± S.E. MG / CAGE / DAY	
	Before drug	**3 days after drug**
Thymoquinone	1.46 ±0.02	2.4 ±0.08*
Carbonyl fraction (polythymoquinone)	1.4 ±0.05	2.6 ±0.07*
Control	1.33 ±0.05	1.25 ±0.01

*Significant change

TABLE II

URIC ACID EXCRETION IN RATS BEFORE, DURING AND AFTER INTRAMUSCULAR ADMINISTRATION THE DRUGS (4mg/kg) AND PROPYLENE GLYCOL (0.1ml)

DRUG	AVERAGE URIC ACID (mg / cage / day) FOR 5 SUCCESSIVE DAYS														
	BEFORE TREATMENT					DURING TREATMENT					AFTER TREATMENT				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Thymoquinone	1.4	1.6	1.5	1.4	1.4	1.5	2.8	2.4	2.3	1.8	1.4	1.5	0.9	0.95	1.0
Carbonyl fraction	1.6	1.4	1.5	1.3	1.2	2.1	2.4	2.6	2.2	1.8	1.6	0.7	0.58	0.25	0.7
Control	1.6	1.5	1.3	1.0	1.25	1.5	1.55	1.25	0.88	1.25	1.0	0.88	0.75	1.0	1.35

TABLE III

EFFECT OF THE CARBONYL FRACTION AND THYMOQUINONE (1mg/kg) ON THE EXCRETION OF BILE IN DOGS

DRUG	AVERAGE BILE EXCRETED IN 30 MINUTES ± S.E.			
	BEFORE DRUG		30 MINUTES AFTER DRUG	
	Volume in ml	Total solid in gm	Volume in ml	Total solid in gm
Thymoquinone (10 dogs)	1 ±0.03	0.12 ±0.01	2 ±0.06	0.14 ±0.02*
Carbonyl fraction (10 dogs)	1.6 ±0.05	0.09 ±0.015	2 ±0.05	0.11 ±0.01*
Control (5 dogs)	2.2 ±0.10	0.11 ±0.01	2 ±0.04	0.093 ±0.01

* Significant change.

TABLE IV

THE EFFECT ON INHALATION OF HISTAMINE MIST[1] IN GUINEA PIGS RECEIVING AN INTRAPERITONEAL INJECTION OF THYMOQUINONE OR THE CARBONYL FRACTION. CONTROL ANIMALS WERE INJECTED WITH PROPYLENE GLYCOL (0.1 ml).

No. of Experiment	TIME (IN MINUTES AND SECONDS) ELAPSING BEFORE THE ONSET OF CONVULSIONS		
	Control Animals	Thymoquinone 80 mg/kg	Carbonyl Fraction 50 mg/kg
1.	1'20"	C.P.(2,3)	C.P.
2	1'10"	C.P.	C.P.
3.	55"	C.P.	C.P.
4.	1'08"	7'45"	C.P.
5.	1'07"	C.P.	C.P.
6.	1'0"	C.P.	C.P.
7.	1'12"	C.P.	6'30"
8.	1'05"	C.P.	C.P.
9.	1'17"	C.P.	C.P.
10.	1'05"	C.P.	C.P.
11.	1'08"	C.P.	C.P.
12.	1'	C.P.	C.P.
13.	1'07"	C.P.	C.P.
14.	1'12"	C.P.	C.P.
15.	1'17"	C.P.	C.P.

(1) 0.25% aqueous histamine acid phosphate; sprayed under a pressure of 0.5 kg/sq.cm.

(2) C.P. complete protection, i.e. no obvious signs of convulsions in 10 mins.

(3) All animals injected with thymoquinone died in the course of few hours.

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POSSIBLE EFFECT OF SOME EXTRACTS OF NIGELLA SATIVA SEEDS ON BLOOD COAGULATION SYSTEM AND FIBRINOLYTIC ACTIVITY

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POSSIBLE EFFECT OF SOME EXTRACTS OF NIGELLA SATIVA SEEDS ON BLOOD COAGULATION SYSTEM AND FIBRINOLYTIC ACTIVITY*

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INTRODUCTION

Nigella sativa L. seeds have been reported to possess many pharmacological effects. It has been reported that the Prophet Mohammed (ﷺ) said that the black seed is a remedy for every disease except death (al-Jawzeyah)[1]. Nigellone, an active principle isolated from *Nigella sativa* was shown to possess a protective effect in guinea pigs against histamine induced bronchospasm and was found to be free of toxic effects (Mahfouz and el-Dakhakhny, 1960)[2]. Fractions from *Nigella* seeds were used therapeutically in the treatment of bronchial asthma in adults (Mahfouz *et al*, 1960)[3]. The Nigella was also found to possess antibacterial effect (Toppozada *et al*, 1964)[4].

It was claimed, by some people in Kuwait, that in certain cases of epistaxis, a preparation obtained from *Nigella* seeds was useful in the management of such cases. The people extracted the crushed *Nigella* seeds with a natural fat, few drops of the product were instilled in the nostrils, few minutes later, bleeding ceased. In addition, they reported that no recurrences of bleeding were observed after several times of application of the drug.

MATERIALS AND METHODS

Male rabbits obtained from local breeding were used throughout this work. The animals were kept under the same conditions,

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given food and water *ad libitum*. The animals were classified into 2 main groups, the first was used for the *in vitro* study. The second group was used for the *in vivo* study. A control group was used with each test group and procedures of both control and test were done simultaneously.

Blood was obtained from the orbital plexus of the rabbit using a capillary tube.

Drugs

1) The fatty extract of *Nigella sativa* seeds (Yasseen, 1981)[4]: The *Nigella sativa* seeds were crushed. Fresh natural fat (ghee) was used to extract the seeds in a concentration of 1.143kg ghee for every 100gm of the crushed seeds. The extraction of the seeds was carried out by heating the fat with the crushed seeds for 20 minutes (on a water bath). The extract was then filtered through a thick muslin (Fraction I). The fatty extract when melted again was separated into two layers, an upper fatty layer (Fraction II) and a lower aqueous layer (Fraction III). The natural fat used for extraction (Fraction IV) was tested for any possible effect on coagulation system. All of these fractions are fatty in nature. In order to be mixed with blood or plasma, they were emulsified with tween 80. The effect of the emulsifying agent (Fraction V) alone was also studied on blood coagulation system.
2) The petroleum ether extract of *Nigella sativa*: 250gm of *Nigella sativa* seeds were crushed and extracted with petroleum ether (40/60). Eighty gram of extract was obtained.

PREPARATION OF THE WORKING SOLUTIONS OF THE DRUGS

Fraction I

The whole fatty extract was emulsified with tween 80. 5gm of extract was emulsified with 2ml tween 80 and completed to 100ml with saline. The working solution was obtained by diluting this fraction with saline (1:10 V/V).

Fraction II

The whole fatty extract was melted, the upper fatty layer was separated and emulsified with tween 80 in a concentration of 2 gm with 1ml tween 80 and completed to 100ml with saline. The working solution was obtained by diluting this solution with saline (1:10 V/V).

Fraction III

The lower aqueous layer of the fatty extract was separated and diluted with saline (1:10 V/V).

Fraction IV

The natural fat (extracting agent) was emulsified with tween 80 in a concentration of 5gm with 2ml tween 80 and completed to 100ml with saline. The working solution was diluted with saline (1:10 V/V).

Fraction V

The emulsifying agent tween 80 was mixed with saline in a concentration of 2ml diluted to 100ml. The working solution was diluted with saline (1:10 V/V).

Fraction VI

The petroleum ether extract was emulsified with tween 80 in a concentration of 2 gm with 1 ml tween 80 and diluted with saline to 100ml. The working solution was diluted with saline (1:10 V/V).

The *in-vitro* study

In the *in-vitro* study the working solutions were mixed with plasma or blood, in a concentration of 10% V/V, and incubated for 2 minutes.

The *in vivo* study

The petroleum ether extract was prepared for injection to the animals. Two grams of the petroleum ether extract was emulsified with 1 ml tween 80 and completed to 100 ml with saline. Male rabbits were slowly injected with this solution through the ear vein in a dose equivalent to 10 mg of the petroleum ether extract per kg body weight daily for 7 days. Withdrawal of blood samples for different tests was carried out 24 hours after the last injection.

Procedures

The preparation of platelet rich plasma (PRP) and platelet poor plasma (PPP) was done according to Owen *et al* (1975)[5].

Procedures included the following:

1) Whole blood clotting time (Dacie & Lewis, 1975)[6].
2) Plasma clot time (Austen & Rhymes, 1975)[7].
3) Kaolin cephalin clotting time (Austen & Rhymes, 1975)[8].
4) Prothrombin time (Austen & Rhymes, 1975)[9].
5) Thrombin time (Austen & Rhymes, 1975)[10].
6) Stypven time (Austen & Rhymes, 1975)[11].
7) Euglobulin clot lysis time (Austen & Rhymes, 1975)[12].
8) Partial thromboplastin time (Nye *et al*, 1962)[13].
9) Bleeding time (Thienes *et al*, 1957)[14].

In this test, male white albino rats weighing about 150gm each were used. The rats were anaesthetized with thiopentone sodium in a dose of 6 mg/100 gm body weight intraperitoneally. The abdomen was opened and the liver was gently lifted out. A piece of liver was cut from a portion of the edge with sharp scissors, leaving a cut surface of about 10mm long and 3-4mm wide. The drugs were applied to the cut surface, the bleeding time was then determined. The length of bleeding time was determined by gently blotting with pieces of filter paper at 10 seconds intervals. The end point was sharp and indicated by a blood clot clinging to the filter paper, but with little or no liquid blood wetting it. The significance of the difference of the mean from that of the control group and from those treated groups, was determined by calculation of the critical ratio.

The critical ratio $$\frac{-X_1 - -X_2}{\sqrt{\frac{(SE_1)^2}{N_1} + \frac{(SE_2)^2}{N_2}}}$$

where X_1 and X_2 are the mean bleeding times being compared, SE_1 and SE_2 are the standard errors of X_1 and X_2, and N_1 and N_2 are the number of animals used.

The differences are considered significant if the critical ratio equals 2 or more than 2.

RESULTS

In the *in vitro* study, the petroleum ether extract (Fraction VI) and the upper layers of the fatty extract of *Nigella* produced statistically significant shortening in the whole blood clotting time. On the plasma clotting time, all fractions produced significant shortening when compared to control group but only Fractions I, II and VI produced significant shortening in this parameter when compared to the group of emulsifying agent (Fraction V). Only the petroleum ether extract (Fraction VI) produced significant shortening in the Kaolin cephalin clotting time when compared to either control group or emulsifying agent group.

On the stypven time, the petroleum ether extract (Fraction VI) and the upper layer of fatty extract (Fraction II) produced a significant shortening when compared to the emulsifying fraction group (Fraction V). It was found that the emulsifying group produced a significant prolongation in the stypven time when compared to the control group. On the euglobulin clot lysis time the petroleum ether extract (Fraction VI), Fraction I and Fraction III produced significant prolongation in this parameter (Table I).

The petroleum ether extract and the whole fatty extract of *Nigella* produced a significant shortening in the bleeding time in the rat as indicated by the increase induced in the critical index by both fractions (Table II).

In the *in vivo* study, the petroleum ether extract produced significant shortening in the whole blood clotting time, plasma clotting time and Kaolin cephalin clotting time. No significant effect was observed on the prothrombin time or thrombin time. There was significant shortening in the partial thromboplastin time. The euglobulin clot lysis time was significantly prolonged (Table III).

TABLE I

THE *IN VITRO* EFFECT OF DIFFERENT FRACTIONS OF *NIGELLA SATIVA* ON SOME BLOOD COAGULATION PARAMETERS AND FIBRINOLYTIC ACTIVITY

	Control	Fraction I** (The whole fatty extract)	Fraction II (The upper layer of the fatty extract)	Fraction III (The Aqueous phase of the fatty ext.)	Fraction IV (The extracting fatty substance alone)	Fraction V (The emulsifying agent)	Fraction VI (The petroleum ether extract)
Whole blood* clotting time	416.4 ± 71.6 (7)[c]	337.5 ± 14.7 (8)	220.6 ± 20.5[ab] (9)	360.0 ± 62.5 (9)	183.3 ± 21.7 (9)	491.1 ± 89.1 (9)	141.7 ± 41.9[a,b] (9)
Plasma clot time	124.6 ± 5.9 (19)	79.6 ± 3.9[ab] (12)	82.0 ± 5.3[a,b] (10)	91.0 ± 7.6[a] (6)	86.6 ± 7.6[a] (10)	100.0 ± 5.7[a] (8)	64.4 ± 6.8[a,b] (10)
Kaolin Cephalin clot time	46.7 ± 3.4 (14)	45.3 ± 4.3 (9)	46.5 ± 5.6 (6)	40.7 ± 2.7 (6)	48.3 ± 4.1 (9)	45.0 ± 6.9 (8)	28.4 ± 1.99[a,b] (7)
Stypven time	14.9 ± 2.6 (16)	16.1 ± 1.8[a] (11)	10.7 ± 0.5[a,b] (12)	16.9 ± 1.7 (11)	14.7 ± 1.0 (10)	21.7 ± 2.3[a] (12)	12.8 ± 1.03[b] (11)
Euglobulin clot lysis time	87.4 ± 7.6 (10)	186.3 ± 49.1[a,b] (8)	107.5 ± 12.3 (8)	126.6 ± 12.9[a,b] (9)	96.30 ± 8.7 (8)	82.3 ± 16.8 (8)	227.5 ± 51.6[a,b] (8)

* Figures represent the mean ± S.E.

** Figures are in terms of seconds except the euglobulin clot lysis time which is in minutes

a. Statistically significant when compared to control group

b. Statistically significant when compared to the emulsifying group

c. Figures represent the number of animals.

TABLE II

EFFECT OF THE FATTY EXTRACT AND PETROLEUM ETHER EXTRACT OF *NIGELLA SATIVA* ON BLEEDING TIME IN THE RAT

	Control	Fatty extract	Pet. ether extract
Mean ±*	180.7±	95.6±**	151.3±**
S.E.	12.2	6.3	16.9
n	(22)[a]	(9)	(12)
Critical index	-	25.49	5.32

* time in terms of seconds

** statistically significant according to Thiens equation

a Figure between brackets represents the number of animals used

TABLE III

EFFECT OF ADMINISTRATION OF THE PETROLEUM ETHER EXTRACT TO RABBITS ON SOME BLOOD COAGULATION PARAMETERS AND FIBRINOLYTIC ACTIVITY

	Whole blood clotting time (seconds)	Plasma Clot time (seconds)	Kaolin Cephalin clot time (seconds)	Prothrombin time (seconds)	Thrombin time (seconds)	Partial Thrambo-plastin time (seconds)	Euglobulin time (seconds)
Control	355.3 ± 55.36 (6) a	117.4 ± 7.83 (21)	54.6 ± 3.7 (27)	14.0 ± 0.49 (20)	18.5 ± 0.97 (27)	58.3 ± 0.00 (6)	151.2 ± 40.1 (6)
Treated	248.3 ± 19.9 (10)	84.30 ± 4.3 (38)	45.8 ± 2.4 (42)	13.0 ± 0.42 (25)	17.2 ± 0.92 (28)	40.1 ± 0.00 (11)	307.5 ± 42.6 (6)
% change fram control	-30	-28.2	-16.1	-7.1	-7.0	-31.2	+ 102.1
P	< 0.001	< 0.001	< 0.05	> 0.05	> 0.05	< 0.05	< 0.05

(a) Figures between brackets represent the number of animals

DISCUSSION

The fatty extract of *Nigella sativa* seeds was reported by many people to stop bleeding in some cases of bleeding nose (epistaxis). This work was undertaken to investigate the possible effect of such a preparation on blood coagulation system.

The problem was complicated by the fact that the material consisted of the extract in addition to the fatty solvent. The extract itself contains several components. It was also planned to study the effect of such fatty extract in comparison to the petroleum ether extract of the *Nigella sativa* seeds. The petroleum ether extract was reported to contain most of the active ingredients of *Nigella* seeds (Gad *et al*, 1963[15], El-Dakhakhny, 1963[16]). The petroleum ether extract and the upper layer of the fatty extract were shown to shorten the whole blood clotting time. This suggests that the fatty solvent may extract from the *Nigella* seeds some fatty soluble constituents that affected the blood clotting time. The petroleum ether extract and the fatty extract were emulsified with tween 80, in order to make them miscible with plasma or blood. The effect is not due to the emulsifying agent. The shortening in wholeblood clotting time could be due to, at least partially, a coagulant effect. Coagulants accelerate the clotting of normal and some pathological blood both *in vivo* and *in vitro* (Coss, 1964)[17]. The petroleum ether extract (*in vivo* and *in vitro*) and the fatty extracts of *Nigella (in vitro)* produced significant shortening in the plasma clot time. Again, the effect is not due to the emulsifying agent and not due to the fatty solvents as shown in Table I. The effect is not mediated through the aqueous phase of the fatty extract.

The mechanism of action is non specific, the effect could be through one or more of the clotting factors. This test, involves the whole blood clotting process and thereby measures most coagulation factors (Miale, 1972)[18]. The observations may also indicate that the effect of such extracts may be induced, at least partially, through the intrinsic mechanism of blood coagulation (Owen *et al*, 1975)[19].

The fatty extract and petroleum ether extracts of *Nigella* produced a shortening in the bleeding time. The bleeding time depends on a number of factors. Haemostasis in wounds measuring the bleeding time depends upon the rate at which a stable platelet thrombus is formed and thus measures the efficiency of the vascular and platelet phases (Tocantins, 1936)[20].

The petroleum ether extract (*in vitro*) produced shortening in the Kaolin cephalin clotting time. This test detects the intrinsic procoagulation activity of plasma except platelet factor III, factor XIII and factor VIII (Owen *et al*, 1975)[21]. The petroleum ether extract can stimulate or enhance the activity of one or more of the factors sensitive by such a test. The petroleum ether extract produced the same effect when given intravenously.

The fatty extract and petroleum ether extract (*in vitro*) produced a significant decrease in the stypven time. The Russel's viper venom (stypven) is known to have thromboplastic activity when added to recalcified plasma (Miale, 1972)[22].

The effect could be due to an action on platelets, or possibly due to the fatty nature of the extracts. It was reported that thrombocytosis and various hyperlipidemic states tend to be associated with an abnormally short stypven time (Owen *et al*, 1975)[23].

The fatty extract and petroleum ether extract inhibited the fibrinolytic activity *in vivo* and *in vitro*. The mechanism of this effect is not known but it may be due to an inhibitory effect on the activation of plasminogen or inhibition of the preformer plasmin activity. The drugs having antifibrinolytic activity such as epsilon amino caproic acid and tranxamic acid were reported to be used successfully in epistaxis (Hardy, 1974)[24]. They also could improve the effectiveness of therapy in haemophilia following dental extraction (Reid *et al*, 1974[25], Walsh *et al*, 1971)[26]. Inhibition of fibrinolytic activity may be advantageous even though the basic disease may have other causes.

The extracts of *Nigella sativa* seeds have some effects on the blood coagulation and fibrinolytic activity. The claim that the fatty extract was used successfully by the public in certain cases of epistaxis has a certain degree of reality. The fatty solvent may play a mechanical role in stopping bleeding. Other possible mechanisms may share in this effect. Chemical investigation of the constituents of *Nigella* should be carried out and further investigations are needed to complete the picture.

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PHARMACOLOGICAL EVALUATION OF BERBERIS ARISTATA IN EXPERIMENTAL CHOLERA AND OTHER DIARRHOEAS

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INDIA

INTRODUCTION

Exhaustive description of the medical usefulness of the plant *Berberis aristata* (Arabic - "Ambarbaris"; "Aargis") has been given by Bu-Ali-Seena[1]. This plant has been claimed to be effective in bleeding disorders, haemorrhoids, flatulance, dysentery, indigestion, sprue, anal fissure, diarrhoea and gastroenteritis and as stomachic, choleretic, antispasmodic and constipatory. Utility of this plant has also been appreciated by other Arab Physicians[2]. The value and popularity of this plant has persisted upto the twentieth century since several formulations developed for the treatment of acute gastroenteritis, diarrhoea and dysentery contain *Berberis aristata* decoction/extract ("Aargis") or its alkaloid berberine as one of the important ingredients.

Berberine ($C_{20}H_{19}O_5N$) is the chief alkaloid present in *Berberis aristata*. Scientific evidence of its empirical use in gastroenteritides came only in 1967 when its efficacy was demonstrated in clinical trials in patients suffering from cholera or severe nonspecific diarrhoea; it was observed that altogether berberine was more effective than chloramphenicol[3]. The present study was, therefore, planned to study its effect in experimentally-induced cholera and

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other diarrhoeas and to work out the possible mechanism of antidiarrhoeal effect of berberine.

MATERIAL AND METHODS

Cholera toxin

Crude cholera toxin powder was manufactured by the Wyeth Laboratories, Marietta, U.S.A. The toxin was received in two lots, namely, Lot 001 prepared from *Vibrio cholerae* strain B1307 and Lot 002 from the strain B569. However, in the following experiments, they did not show difference in their potency. Therefore, they have not been mentioned individually and the crude cholera toxin powder of both the lots is just referred to as "cholera toxin". Each gramme of crude powder contained 1.068 mg of highly purified cholera toxin (Miller, 1974, Personal communication). This highly purified toxin is also known as "fluid accumulating factor (FAF)" or "choleragen" or "enterotoxin" or "cholera enterotoxin". Aqueous solution of the cholera toxin was prepared freshly every day for our experiments.

Effect of drugs on diarrhoea induced by feeding cholera toxin in adult rats

Albino rats (112-258gm) of either sex were denied food for 6 hours but were allowed water *ad lib.* Thereafter, the drugs were administered through a stomach tube. To different groups of rats, berberine (sulphtate), Aargis (the traditional crude dried preparation of *Berberis aristata* decoration), atropine (sulphate), morphine (sulphate), indomethacin (suspended in propylene glycol) or distilled water was administered, orally, 5 minutes before cholera toxin. Individual rats were then placed in separate metallic cages with blotting paper spread at the bottom. Water was allowed throughout 24 hours observation period but food was denied for 7 hours after giving cholera toxin. The latent period of diarrhoea and the number of loose stools passed by individual rats were noted.

Purging response of each group was comprehensively assessed by calculating the purging index as per the following formula -

$$\text{Purging Index} = \frac{\text{Per cent rats responding by purging x Mean number of loose stools of respondents}}{\text{Mean latent period of respondent in hours}}$$

Effect of drugs on cholera toxin-induced fluid formation in the gastrointestinal tract of adult albino rats

Animals (105-200gm) were denied food for 6 hours but given water *ad lib.* Groups of rats received the drugs, orally, about 5 minutes before cholera toxin. Individual rats were then placed in separate cages with water but without food and were observed for 4 hours. As soon as an individual passed a loose stool, it was killed by chloroform; those which did not pass loose stools were killed exactly at 4 hours. The abdomen was opened, gastrointestinal tract dissected out and its contents carefully collected, measured and weighed. Water value was calculated from the fresh and constant dry weights of the contents. In many experiments, after ascertaining the constant dry weight, the dried residue was thoroughly mixed with 75ml distilled water and the clear supernantant was used for estimating Na^+ and K^+ (by flame photometer) and chloride[4].

Effect of drugs on the cholera toxin-induced fluid accumulation in the ligated intestinal loop of adult rats

The method[5] was modified as follows to render it more consistent. Adult albino rats (118-185gm) of either sex were denied food for 27-36 hours but had 5 per cent glucose water *ad lib.* Abdomen was opened under pentobarbitone anaesthesia and, extending from 3cm below the pylorus, a 30cm long intestinal loop was prepared by two ligatures. Berberine, atropine, morphine, indomethacin (suspended in propylene glycol), propylene glycol or distilled water was injected into the loop just before cholera toxin injection. Loop fluid was collected after 5 hours and its Na^+, K^+ and Cl^- concentrations were estimated. In view of the possibility

that berberine might act against cholera toxin promptly or after many hours, in some groups, loop fluid was collected at 3, 8 or 12 hours after injecting berberine and cholera toxin.

Effect of berberine on diarrhoea induced by *Ipomoea turpethum* root powder in dogs

Healthy mongrel dogs (8-16kg) of either sex were maintained on boiled meat, bread and water *ad libitum*, for atleast 3 days; only those dogs which passed formed stools were used. For inducing diarrhoea[6], 1gm of freshly powdered root of *Ipomoea turpethum* (Arabic - "Turbud") was mixed with 200ml milk and dogs were allowed to drink. In some experiments, berberine was given with *Ipomoea* powder in the milk. Negative control animals received only milk. To assess severity of diarrhoea, frequency of defaecation and consistency of each stool passed by individual dogs were observed over 24-hour period. Stools were classified into the following categories and assigned grades: solid formed stool-1 grade; soft uniform stool - 2 grades; and frank liquid stool - 4 grades. Grades secured by each dog over a 24-hour period after drugging were recorded.

Effect of berberine on magnesium sulphate-induced diarrhoea in adult rats

Adult albino rats (100-190gm) of either sex were denied food for 6 hours but were allowed water *ad libitum*. Magnesium sulphate ($MgSO_4.7H_2O$) was administered orally, by stomach tube, in doses of 1.25, 2.5 and 5gm/kg as 25 per cent w/v aqueous solution. Individual rats were then placed in separate cages which had white blotting paper spread at their bottom. Rats were watched for 8 hours for the latent period of diarrhoea and number of loose stools. Water (without food) was freely available throughout this period. Severity of diarrhoea of each group was comprehensively assessed by calculating the purging index. Effect of berberine on diarrhoea induced by 5gm/kg dose of magnesium sulphate was studied in

some rats; in this experiment, berberine was administered about 5 minutes before magnesium sulphate.

Intestinal motility of mice by the charcoal meal method

Adult mice (15-30gm) of either sex were denied food for 24 hours but offered 5 per cent w/v glucose water *ad libitum*. Charcoal meal[7] was prepared by suspending 1gm finely powdered activated charcoal in 10ml of about 25 per cent gum acacia in water. The mice were given orally 0.2ml of the charcoal meal, 30 minutes after they received the following drugs (intraperitoneally) - berberine, atropine, morphine, carbachol, neostigmine or distilled water. In some groups berberine, atropine or morphine was given by the oral route. Effect of giving berberine orally, thrice, at an interval of 2 hours was also studied in some mice. Twenty minutes after charcoal meal feeding, mice were killed by sharp blow on the head. The abdominal cavity was opened and the entire small intestine from pylorus to ileocaecal junction was then gently freed by cutting the intestinal edge of the mesentery. The freed intestine was gently placed, without stretching, in a straight line on a white filter paper. Length of the entire small intestine as also of the portion traversed by dark-coloured charcoal meal were measured. Percentage of the small intestine length travelled by charcoal was then calculated.

Study of the alluded astringent action of berberine

Purified bovine serum albunin (0.5 per cent w/v in water) or fresh egg-white (25 per cent v/v in water) was taken in 1-2ml volumes, in a series of clean glass test tubes. To these tubes, different quantities of berberine or tannic acid dissolved in distilled water were added and the degree of precipitate formation was observed. Solubility of precipitates in 0.1 N KOH or alcohol was also studied.

Passage of electrolytes into 5.5 per cent glucose water injected into the rat peritoneum

Adult albino rats (90-140gm) of either sex were anaesthetized by intraperitoneal injection of sodium pentobarbitone (35mg/kg).

The animals were injected[8] intraperitoneally 5ml glucose water (5.5 per cent) per 100 gm body weight. One ml of the injected fluid was aspirated from the peritoneal cavity every 5 minutes upto 20 minutes and subsequently after 30 minutes and thereafter every 30 minutes upto 120 minutes. The volume of peritoneal fluid drawn on each occasion was replaced by an equal volume of glucose water. The aspirated peritoneal cavity fluid samples were then used for estimation of Na^+, K^+ and Cl^-. In some experiments, different doses of berberine were mixed with 5.5 per cent glucose water before it was injected intraperitoneally.

RESULTS

Effect of berberine on diarrhoea induced by feeding cholera toxin in adult rats

Results are presented in Table 1. For about 3 $\frac{1}{2}$ hours after oral administration of cholera toxin, rats passed a few well-formed pellets which were not counted. Subsequently, they passed soft to frank liquid stools which are referred to as "loose stools". Most of the animals recovered completely within 7 hours after feeding cholera toxin; there was no mortality or apparent distress to the animals. On feeding 2gm/kg cholera toxin, rats manifested diarrhoea (average loose stools 4.9) and showed high purging index (127). Three mg/kg dose of berberine extended the latent period of diarrhoea. Ten mg/kg dose was more effective in that it also reduced the average number of loose stools from 4.9 to 3.4 and percentage of respondents from 95 to 50; these factors reduced the purging index to 25. Thirty mg/kg berberine and 1.2gm/kg "Aargis" were also effective. Four gm/kg dose of cholera toxin produced more severe purging but no mortality; it was reduced convincingly by 10 and 30 mg/kg dose of berberine. In this method atropine and morphine proved more effective than berberine. Indomethacin was entirely ineffective.

Effect of berberine on the cholera toxin-induced fluid formation in the gastrointestinal tract of adult rats

Results are presented in Table 2. Cholera toxin promoted passage of water and electrolytes into the gastrointestinal tract while berberine and "Aargis" inibited this effect. Thus, rats receiving 2gm/kg cholera toxin had about 3 times (11.9ml) more fluid in the gastrointestinal tract than the control animals (3.9ml). At 10 and 30mg/kg doses berberine significantly reduced this action of cholera toxin. "Aargis" 1.2gm/kg also significantly reduced fluid accumulation though its effect was less than that of 10mg/kg berberine. Berberine as also "Aargis" reduced Na^+ and Cl^- (but not K^+) contents of the gastrointestinal fluid augmented by cholera toxin (Table 2). In this respect, "Aargis" appeared to act better than berberine particularly against chloride loss.

Atropine and morphine did not reduce the fluid accumulating action of cholera toxin though they symptomatically suppressed purging in intact rats. Indomethacin was also totally ineffective in reducing volume and electrolytes.

Effect of berberine on the cholera toxin-induced fluid accumulation in the ligated intestinal loop of adult rats

Results are presented in Table 3. Cholera toxin induced fluid accumulation in the ligated intestinal loop of adult rats; 100mg produced significantly more fluid than 30mg ($P < 0.02$). Five mg berberine convincingly reduced the fluid accumulating action of both the doses of cholera toxin ($P < 0.001$). Thirty mg berberine appeared about as effective as 5mg. At 5mg dose, indomethacin, unlike berberine, proved much less effective whereas at 30 mg dose it was as effective as berberine. Propylene glycol (used for suspending indomethacin) did not inhibit fluid formation. Also, atropine and morphine did not influence the fluid accumulation induced by cholera toxin.

When allowed to act against cholera toxin for different periods, berberine showed clear inhibitory effect during the first 3-5 hours period. After 5-8 hours, there was a general tendency for the fluid to get reabsorbed which was apparently facilitated by berberine.

In this experiment, berberine or other drugs did not influence the electrolyte composition of the fluid accumulated by cholera toxin.

Effect of berberine on diarrhoea induced by *Ipomoea turpethum* ("Turbud") root power in dogs

Feeding of *Ipomoea* powder (1gm/kg) induced prompt purging which usually started within 90 minutes and lasted for 2-3 hours. The stools were profuse, watery and often bile-stained. There was no vomiting or anorexia and the animals remained otherwise normal. Berberine (0.06-20mg/kg) significantly prolonged the latent period and frequency of purging and, thus, significantly lowered the purging score (Table 4). However, antidiarhoeal effect of berberine was not dose-dependent.

In control dogs, 200ml milk alone or alongwith 6 or 20mg/kg berberine did not produce any purging.

Effect of berberine on magnesium sulphate-induced diarrhoea in adult rats

Oral administration of magnesium sulphate produced dose-dependent diarrhoea (Table 5). However, its 5gm/kg dose was found to be most effective as it produced purging in all the rats. Purging started after about 4 hours and subsided within 8 hours, after which the rats started passing normal stools. The average number of loose stools was 5.3. Throughout the experiment, the rats appeared normal and there was no mortality. Berberine in doses of 0.3, 3 and 30mg/kg did not significantly reduce diarrhoea induced by magnesium sulphate. It was observed that in these experiments, availability of water is absolutely essential. In preliminary experiments in which water was not kept in the cages

rats receiving 5gm/kg magnesium sulphate died in a few hours and often before manifesting diarrhoea.

Effect of drugs on the intestinal motility of mice

By intraperitoneal route: As shown in Table 6, in control mice, 60 per cent length of the small intestine was travelled by charcoal. Small doses (1 and 3mg/kg) of berberine had no effect on the motility; 10mg/kg reduced the motility so that charcoal travelled 17 per cent length. Atropine (10mg/kg) and morphine (10mg/kg) had about the same degree of inhibitory effect. Atropine and berberine combination did not manifest a clear additive effect. Neostigmine (100ug/kg) and carbachol (50ug/kg) somewhat increased the motility, the charcoal travelling 74 and 76 per cent length respectively. Berberine, like atropine and morphine, clearly antagonised the stimulant effect of neostigmine and carbachol.

By oral route: In 10 control mice, 65 per cent length of the small intestine was travelled by charcoal. On oral administration of 10mg/kg of berberine, the mean length of charcoal travel was 64 per cent of small intestine (12 mice). Larger dose of berberine (40mg/kg; 10 mice) given orally 2 hours before charcoal feeding, reduced the charcoal travel distance from 65 to 45 per cent. Three administrations of 40mg/kg dose of berberine did not produce any better effect (45 per cent length travelled in 10 mice). When given orally, atropine (10mg/kg; 10 mice) had no effect on the intestinal motility. Morphine (10mg/kg; 10 mice) was more effective by oral route and reduced charcoal travel distance to 38 per cent.

Study of alluded astringent action of berberine

Tannic acid (0.15 - 6mg/ml) precipitated purified bovine serum albumin and fresh egg-white. Density of precipitate was proportional to the amount of tannic acid; 0.1 N KOH but not alcohol immediately and completely dissolved the precipitate. However, berberine (0.15-6mg/kg) did not form a precipitate with bovine serum albumin or egg-white.

Passage of electrolytes into the 5.5 per cent glucose water injected into the rat peritoneum

Results are presented in Table 7. In control animals, considerable amount of Na^+, K^+ and Cl^- appeared into the peritoneal glucose fluid within 5 minutes of its injection and, over 2 hours the values steadily rose. Addition of 0.3, 3 or 30mg/kg berberine did not influence the temporal course or degree of these electrolyte shifts.

DISCUSSION

Berberine convincingly reduced the severity of diarrhoea induced by cholera toxin. It manifested antidiarrhoeal effect in 3 ways; (a) it reduced the percentage of rats that manifested purging, (b) it prolonged the latent period of diarrhoea and (c) it reduced the number of loose stools. These factors, together, decreased the purging index of berberine treated groups.

Berberine also significantly reduced the volume of water and electrolytes that accumulated in the gastrointestinal tract after feeding cholera toxin.

Berberine decisively reduced the fluid accumulation induced by cholera toxin in the rats ligated intestinal loop. These findings in rats agree with the earlier report on adult rabbit ligated ileal loop[9].

Berberine inhibited fluid accumulation by cholera toxin during the first 5 hours. This suggested that the process of late reabsorption of the fluid in the ligated loop is also facilitated by berberine. These findings indicate that berberine may help in clinical cholera by a dual mechanism, namely (a) by inhibiting initial fluid formation and (b) by promoting later reabsorption.

Fluid that accumulates in the ligated rat intestinal loop after cholera toxin instillation shows remarkably constant electrolyte composition in different experimental conditions. Berberine which certainly reduced its volume (Table 3) did not influence electrolyte concentrations. These data suggest that this method is suitable for

studying volume but not electrolytes unlike method 2 which allows study of both fluid and electrolytes.

In the above mentioned 3 methods as also in the previous work[10] involving adult rats, berberine produced its beneficial effects when administered just 5 minutes before or with cholera toxin. In the infant rabbits, on the other hand, berberine had to be given 18-24 hours earlier to elicit action against cholera toxin which prompted earlier workers[9] to propose a role of host tissues. This difference between the two studies could be due to differences in age and species of the animals. Our findings suggest that in adult rats, berberine acts against cholera toxin immediately, directly and perhaps without the role of host tissues.

Over the hundreds of years, "Aargis" - a dark-brown solid has been the common form in which *Berberis aristata* plant has been prescribed and used. It forms a deep yellow suspension when mixed with water. The actual concentration of berberine may vary[11] greatly in different samples of "Aargis" (0-3.5 per cent, average 0.79 per cent). It reduced the severity of diarrhoea and also the fluid volume and electrolytes. Indeed, compared to berberine, "Aargis" appears to inhibit transport of electrolytes more effectively than that of water. It would be interesting to study the contribution of ingredients of "Aargis" other than berberine. The dose of "Aargis" in this work was calculated on its expected berberine content.

Atropine reduces gastrointestinal motility by parasympatholytic action and morphine by a direct potent motor inhibition. Though useful in moderate gastroenteritides, these drugs are considered to be ineffective and even dangerous in severe cholera. The present work supports this clinical opinion. Thus, atropine and morphine apparently manifested anticholera action in that they convincingly reduced the incidence of diarrhoea (Table 1). However, unlike berberine, they did not inhibit fluid accumulation in the gastrointestinal tract (Table 2) or ligated loops (Table 3). Therefore,

it is reasonable to suggest that their diarrhoea-masking effect was entirely due to inhibition of gastrointestinal motility and not due to true anticholera action. This finding also indicates that, during drug screening, anticholera effect obtained in method 1 should be confirmed by methods 2 and 3. It is interesting to note that atropine, morphine and berberine were used here in doses that are respectively about 150, 15 and three times higher than the daily human dose.

Indomethacin is a clinically used potent non-steroidal anti-inflammatory drug. In subacute inflammation induced by subcutaneous injection of cholera toxin (which takes 27 hours to develop and lasts for 3 days), berberine and indomethacin were found equally potent inhibitors[10]. Therefore, it was tried against cholera toxin here in the 3 gastrointestinal methods in adult rats. In these methods where the toxin has to act swiftly and for a shorter time, indomethacin was found to be entirely ineffective (Tables 1 and 2) or much less potent (Table 3) than berberine. Further unlike berberine, the dose of indomethacin used in these methods exceeded (10 times) the safe human dose. It is not clear why indomethacin should convincingly inhibit cholera toxin in subcutaneous tissue but not in the gastrointestinal tract.

Ipomoea turpethum (dark variety of "Turbud" plant) root has been used for many centuries as a purgative in the Unani and Ayurvedic systems of medicine. It contains a purgative resin which resembles that of Jalap[12]. These purgative resins are broadly classified as 'irritants'. They act directly on the small intestine where they produce, like cholera toxin, copious outpouring of fluids, which provokes peristalsis leading to quick, profuse, watery motions. In dogs, the severity of *Ipomoea*-induced diarrhoea is dose-dependant and can be expressed semi-quantitatively[13]. Its

frequency and severity are very significantly reduced by berberine. It is interesting to mention here that castor oil induced diarrhoea in dogs was not at all inhibited by berberine (Sabir and Bhide, unpublished data).

Magnesium sulphate is a classic osmotic saline type of purgative. Its purgative effect in rats was not impressively or consistently reduced by berberine. In this connection, it is of interest to note that, in rats, 2gm/kg cholera toxin and 5mg/kg magnesium sulphate produced about the same degree of diarrhoea; and yet, berberine remarkably reduced the former but hardly influenced the latter.

Altogether, experiments in dogs with *Ipomoea* and castor oil and in rats with magnesium sulphate and cholera toxin indicate that berberine inhibits some but not all types of diarrhoea. Therefore, its antidiarrhoeal action is probably specific and not general.

Does berberine help in diarrhoea by inhibiting gastrointestinal motility? The evidence is certainly conflicting for the following reasons: (i) berberine is a weak cholinesterase inhibitor[14] and cholinesterase inhibitors generally increase the gastrointestinal motility. Indeed, in dogs (Sabir and Bhide - unpublished data) and man[13], large doses of berberine or Aargis are known to exert laxative action; (ii) on the other hand, the present study shows that berberine definitely reduces intestinal motility in mice. This could be due to the direct inhibition of intestinal smooth muscles by berberine which is known to inhibit several types of smooth muscles and their stimulants[14,15]. On the whole, the available data are inadequate to draw valid inference.

Berberine reduces passage of water and Na^+ from blood into the renal tubules[15]. This finding raises the question whether berberine helps in diarrhoea because it inhibits passage of electrolytes across all the cell membranes? However, the present study in rats using the model of Darrow and Yannet[8] clearly shows that

berberine does not inhibit passage of electrolytes from the blood into the glucose solution in the peritoneal cavity.

Earlier studies[16] have suggested that berberine might be helping cholera patients due to probable protein precipitating astringent action. Tannins and other astringents are well-known age old antidiarrhoeal agents. However, the present study clearly shows that, unlike tannins, berberine is not an astringent.

Besides the mechanisms described above, there are others by which berberine is likely to inhibit infective diarrhoeas. These are metabolic inhibition of infective micro-organisms[17,18,19], bactericidal action[18,20,21], inhibition of toxin formation by micro-organisms[21], direct antagonism of the formed toxin at the site of target organs[10], and central antiemetic action; this mechanism is, however, excluded because, in dog, berberine had no chlorpromazine-like antiemetic action against apomorphine-induced vomiting[15].

Altogether, these experiments strongly support the clinical efficacy of *Berberis aristata* in acute gastroenteritis, including cholera, as claimed by Bu-Ali-Seena[1] and other Arab Physicians[2].

SUMMARY AND CONCLUSION

At 10mg/kg dose, berberine sulphate significantly reduced the incidence and severity of diarrhoea induced by 2 or 4gm/kg of cholera toxin in rats; each gm of cholera toxin contained 1.068mg of highly purified cholera toxin. Berberine also reduced the levels of water and electrolytes (Na^+, K^+ and Cl^-) in the gastrointestinal luminal fluid in rats fed with cholera toxin; further, it (5mg) also reduced the cholera toxin (30 or 100mg) - provoked fluid accumulation in the ligated loop (30cm) of the rat intestine. Indeed, crude dried decoction of *Berberis aristata* (Arabic - Aargis; Ambarbaris), 1.2gm/kg, was almost equally effective in reducing cholera toxin-induced diarrhoea and fluid formation in the gastrointestinal tract.

Berberine (0.06-20mg/kg) significantly prolonged the latent period and reduced severity and frequency of *Ipomoea turpethum* (Arabic - Turbud; 1gm/kg) - induced purging in dogs. However, it (0.3, 3 and 30mg/kg) did not affect the diarrhoea induced by magnesium sulphate (5gm/kg). Berberine (10mg/kg) markedly inhibited the intestinal motility in intact mice; intraperitoneal route was more efficacious than the oral one.

Berberine did not precipitate, *in vitro*, bovine serum albumin or egg-white nor altered the shift of Na^+, K^+ and Cl^- from the blood into the peritoneal cavity. Therefore, its anticholera toxin or antidiarrhoea effect cannot be due to any astringent action or due to generalised inhibitory effect on electrolyte movement. These findings, suggest the selective nature of antidiarrhoea action of berberine in gastroenteritis or cholera.

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TABLE 1

EFFECT OF BERBERINE AND OTHER DRUGS ON DIARRHOEA INDUCED BY CHOLERA TOXIN IN AUDLT RATS

Dose (gm/kg) of cholera toxin	Dose/kg of a drug fed orally before cholera toxin	Number of rats used	Number of rats responded by purging	Average latent period of respondents hr-min	Average number of loose stools of respondents	Purging index
2	Nil or distilled water 6 ml	21	20	3 40	4.9	127
2	Berberine 3 mg	7	7	5 19	4.3	81
2	Berberine 10 mg	18	9	6 46	3.4	25
2	Berberine 30 mg	10	5	4 52	3.2	33
2	Aargis 1200 mg	7	3	4 50	3.6	32
2	Atropine 10 mg	6	2	9 00	3.0	11
2	Morphine 10 mg	6	1	8 30	1.0	2
2	Indomethacin 10 mg	10	10	4 10	5.2	125
4	Nil or distilled water 6 ml	8	8	2 05	6.9	345
4	Berberine 10 mg	9	6	4 27	3.6	54
4	Berberine 30 mg	10	6	7 19	3.3	27

TABLE 2

EFFECT OF BERBERINE AND OTHER DRUGS ON WATER AND ELECTROLYTES IN THE GASTROINTESTINAL TRACT IN ADULT RATS FED 2gm/kg CHOLERA TOXIN

Dose/kg of a drug fed orally before cholera toxin	Number of rats	Gastrointestinal Contents				
		Volume in ml average ±S.E. (P values)	Water Content average gm	Na^+ mEq./lit. average ±S.E. (P values)	K^+ mEq./lit. average ±S.E.	Cl^- mEq./lit. average ±S.E. (P values)
Distilled water+ 10-20 ml	14	3.9±0.53	2.96	15*	5*	3*
Nil or distilled water 10 ml	17	11.9±0.82	10.10**	*28±1.14	*5±0.90	*20±2.84
Berberine 10 mg	12	8.05±0.43 (<0.001)	5.81*	*22±5.79 (> 0.1)	*5±1.55	*15±3.96 (> 0.1)
Berberine 30 mg	14	7.0±0.32 (<0.001)	6.15*	*19±3.64 (< 0.5)	*4.5±1.19	*15±4.21 (> 0.1)
Aargis 1200 mg	11	9.08±0.55 (< 0.01)	8.00	18±1.31 (< 0.001)	4±0.33	4±0.48 (< 0.001)
Atropine 10 mg	6	10.92±0.82 (> 0.1)	-	-	-	-
Morphine 10 mg	8	11.32±2.00 (> 0.1)	-	-	-	-
Indomethacin 10 mg	7	11.5±1.57 (> 0.1)	11.15	25±2.66 (>0.1)	4±0.53	21±2.07 (> 0.1)

* Data from 5-7 animals of the respective groups

** Data from 11 animals of the group

+ This group was not given cholera toxin

K^+ Values did not differ significantly

TABLE 3

EFFECT OF BERBERINE AND OTHER DRUGS ON FLUID AND ELECTOLYTE ACCUMULATION BY CHOLERA TOXIN IN THE LIGATED INTESTINAL LOOP

Dose of cholera toxin/ loop	Dose of drug/loop	Number of rats	Fluid in the intestinal loop			
			Volume ml average ± S.E (P values)	Na^+ mEq./lit. average	K^+ mEq-lit. average	Cl^- mEq./lit. average
Nil	Distilled water 1.0ml	6	0	-	-	-
30 mg	Nil or distilled water 1.0 ml	22	5.44 ± 0.17	159	1.3	120
30 mg	Berberine 5mg	17	2.83 ± 0.42 (< 0.001)	149	15.1	114
30 mg	Berberine 30 mg	8	3.10 ± 0.54 (< 0.001)	-	-	119
30 mg	Indomethacin 5mg	11	4.30 ± 0.44 (< 0.02)	-	-	117
30 mg	Indomethacin 30 mg	6	3.10 ± 0.48 (< 0.001)	-	-	116
30 mg	Propyleneglycol 0.3ml	6	4.90 ± 0.66 (> 0.1)	-	-	128
30 mg	Atropine 5mg	6	5.07 ± 0.57 (> 0.1)	153	27.5	-
30 mg	Morphine 5mg	6	6.42 ± 0.35	155	24.9	-
100 mg	Nil or distilled water 1.0ml	6	3.95 ± 0.24 (< 0.001)	158	11.4	127
100 mg	Berberine 5mg	6		156	13.9	-

TABLE 4

EFFECT OF BERBERINE ON DIARRHOEA INDUCED IN DOGS BY FEEDING IPOMOEA TURPETHUM ROOT POWDER

Drugs fed with 200ml milk	Dose/kg	Number of Dogs	Latent period of purging in minutes Mean ± S.E. (P values)	Score of purging over 24 hours Mean ± S.E (P values)
Control (milk alone)	200ml per dog	18	No diarrhoea	1
Berberine	6 mg	6	No diarrhoea	3
Berberine	20 mg	6	No diarrhoea	2.8
Ipomoea	1 gm	26	"88 ± 9	"20 ± 1.4
Berberine + Ipomoea	0.06 mg + 1 gm	6	130 ± 15 (< 0.05)	13 ± 1.7 (< 0.01)
Berberine + Impomoea	0.2 mg + 1 gm	6	155 ± 14 (< 0.001)	10 ± 3.7 (< 0.05)
Berberine + Ipomoea	2 mg + 1 gm	6	210 ± 34 (< 0.01)	13 ± 2.4 (< 0.05)
Berberine + Ipomoea	6 mg + 1 gm	3	230 ± 95	7 ± 1.0
Berberine + Ipomoea	20 mg + 1 gm	16	165 ± 17 (<0.001)	12 ± 1.8 (< 0.001)

"These values were used as standard for calculating P values by the 't' test.

TABLE 5

EFFECT OF BERBERINE ON DIARRHOEA INDUCED IN RATS BY MAGNESIUM SULPHATE

Drugs given orally	Dose/kg	Number of rats used	Number of rats responded by purging	Mean latent period of respondents in min ± S.E.	Mean number of loose stools for the whole group ± S.E. (P value)	Purging Index
Distilled water	20 ml	17	Nil	-	0	0
M_gSO_4	1.25 gm	8	1	330	0.25	4.5
	2.50 gm	8	7	257±25	4.0±0.66	95
	5.0 gm	21	21	"240±20	"5.3±0.31	133
Berberine + M_gSO_4	0.3 mg + 5 gm	10	10	195±29 (> 0.1)	5.2±0.29 (> 0.05)	160
Berberine + M_gSO_4	3 mg + 5 gm	10	10	185±33 (> 0.1)	4.8±0.51 (>0.05)	156
Berberine + M_gSO_4	10 mg + 5 gm	18	18	216±19 (> 0.1)	4.1±0.34 (= 0.01)	114
Berberine + M_gSO_4	30 mg + 5 gm	11	10	274±28 (>0.1)	4.7±0.65 (> 0.05)	103

"Values used as standard for calculating P values by 't' test.

TABLE 6

EFFECT OF BERBERINE AND DRUGS ON THE INTESTINAL MOTILITY OF MICE AS ASSESSED BY THE CHARCOAL MEAL METHOD

Drugs injected intraperitoneally	Dose/kg	Number of mice	Mean of the per cent length of small intestine travelled by charcoal meal (range)
Distilled water	10 ml	16	60 (43-81)
Berberine	1 mg	11	63 (50-74)
Berberine	3 mg	8	64 (47-88)
Berberine	10 mg	10	17 (0-42)
Atropine	10 mg	10	14 (4-34)
Morphine	10 mg	10	15 (5-31)
Berberine + Atropine	10 mg + 10 mg	8	12 (0-26)
Neostigmine	100 ug	9	74 (58-90)
Neostigmine + Berberine	100 ug + 10 mg	10	16 (0-42)
Neostigmine + Atropine	100 ug + 10 mg	8	37 (7-73)
Neostigmine + Morphine	100 ug + 10 mg	5	28 (24-33)
Carbachol	50 μg	8	76 (48-100)
Carbachol + Berberine	50 ug + 10 mg	10	38 (18-54)
Carbachol + Atropine	50 ug 10 mg	10	26 (5-41)
Carbachol + Morphine	50 ug + 10 mg	6	13 (0-33)

TABLE 7

TEMPORAL CHANGES IN THE ELECTROLYTE COMPOSITION OF 5.5 PERCENT GLUCOSE SOLUTION INJECTED INTO THE PERITONEUM OF ANAESTHETIZED ADULT RATS

Group of rat	Electrolytes (mEq./L)	Time of collection of peritoneal fluid in minutes							
		5	10	15	20	30	60	90	120
Control, only 5ml glucose water/ 100 gm rat weight	Na^+	43	50	53	64	75	92	90	119
	K^+	2.2	2.5	2.3	2.9	2.2	5.0	5.6	5.6
	Cl^-	28	38	43	50	59	76	89	84
0.3mg/kg berberine in glucose water	Na^+	39	55	67	65	73	-	-	-
	K^+	1.7	2.1	2.5	2.4	2.6	-	-	-
	Cl^-	32	43	53	50	59	-	-	-
3mg/kg berberine in glucose water	Na^+	54	47	74	84	82	80	87	-
	K^+	2.4	1.6	2.5	2.4	3.7	5.3	6.7	-
	Cl^-	27	41	57	52	64	73	84	-
30 mg/kg berberine in glucose water	Na^+	41	55	50	59	64	115	87	121
	K^+	2.6	2.9	2.7	3.3	4.3	5.0	6.7	5.6
	Cl^-	38	52	52	48	69	89	84	89

5-14 animals were used for each value; -, Not done.

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PHARMACOLOGICAL EVALUATION OF ANTIHEPARIN AND ANTITRACHOMA ACTIONS OF BERBERIS ARISTATA

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PHARMACOLOGICAL EVALUATION OF ANTIHEPARIN AND ANTITRACHOMA ACTIONS OF BERBERIS ARISTATA*

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Abstract

The plant *Berberis aristata* has been claimed to be effective in bleeding disorders and in eye diseases. Experiments were, therefore, planned to study the anitheparin and antirachoma actions of berberine - an alkaloid which is abundantly present in this plant.

It was observed that, in dose range of 1-3 mg berberine neutralized, *in vitro*, the anticoagulant action of 50 I.U. heparin per ml of blood and had no effect on blood samples rendered incoagulable by potassium oxalate, sodium citrate and EDTA. Parodoxically, large dose (10mg/ml) of berberine itself produced anti-coagulant effect. These effects resembled those produced by protamine sulphate and toluidine blue.

Berberine protected 50-75 per cent chick embryos from the lethal effect of trachoma organisms inoculated into the yolk sac. It also completely inhibited development of the elementary bodies on the yolk sac membrane. In control experiments, 1 mg per egg dose of sulphadiazine produced similar effect. Further, berberine was found encouragingly effective in controlling experimentally-induced trachoma in monkey eyes.

These actions of berberine strongly support the clinical efficacy of *Berberis aristata* in bleeding disorders and in eye diseases as claimed by Bu-Ali-Sina[1] and Nafisi[2].

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INTRODUCTION

The compilation[3] of the works and writings of Arab Physicians indicates that the plant *Berberis aristata* (Arabic-Ambarbaris; Aargis) has been extensively used in Unani Medicine as emmenagogue, blood purifier, antispasmodic, stomachic, choleretic, sedative, refrigerant, counter-irritant and as a depressant of uterine musculature. Its efficacy in bleeding disorders and in eye diseases has been mentioned by Bu-Ali-Sina[1] and Nafisi[2]. The value and popularity of this plant have persisted upto the twentieth century since several formulations developed for menstrual disorders and eye diseases contain its extract as one of their important ingredients.

Berberine ($C_{20}H_{19}O_5N$) is the chief alkaloid of *Berberis aristata*. Indeed, in its pure form, berberine was clinically tried in patients of chronic trachoma by Varma[4] who found its intra-conjunctival injections highly effective. Incidentally, however, no experimental work has been reported to scientifically vindicate the claim of its usefulness as styptic or in eye diseases. The present work was, therefore, planned to study the antiheparin and antitrachoma actions of the pure alkaloid berberine.

MATERIAL AND METHODS

Experiments on *in vitro* antiheparin action

Blood samples (5-15 ml) collected from the saphenous vein of dogs, jugular vain of goats and bull-calves and cubital vein of human volunteers were quickly distributed, in 1 ml volumes, into a series of dry test tubes (Corning, 1.3 x 10.0 cm) containing heparin (50 I.U.) or EDTA (2-4 mg) or potassium oxalate (3 mg) or sodium citrate (4-10 mg). Berberine sulphate (Unichem Laboratories, Bombay) in doses of 0.3, 1.0, 3.0 and 10.0 mg was added within 10 minutes to the tubes of individual blood samples. For detection of coagulation time, the test tubes were kept at 37°C and gently tilted every minute for the first 10 minutes. During the next 30 minutes,

they were observed at 5 minutes intervals. In most of the experiments, the tubes were kept for occasional observation for about 24 hours.

Corresponding studies using similar doses of standard antiheparin agents - protamine sulphate (Biological Evans, Hyderabad) and toluidine blue (Merck, Bombay) were also conducted. The effect of certain other alkaloids (each in 3 mg doses), namely-atropine sulphate, morphine sulphate, physostigmine sulphate, strychnine hydrochloride, quinidine sulphate and quinine sulphate was also studied to ascertain whether the heparin neutralizing action is exhibited by alkaloids in general or is specific to berberine.

The direct effect of different doses of berberine sulphate, toluidine blue and protamine sulphate on the normal blood clotting time was also investigated.

Experiments on antitrachoma action in chick embryo

Embryonated eggs from the WLH hen maintained on antibiotic free diet were incubated at 37°C in a humidified incubator. The eggs were manually rotated, twice a day, to ensure uniform development of embryo. On day 6 of incubation, the eggs were checked over a 'candling box' for the development of embryo and those showing actively moving embryo with prominently shining blood vessels were used for innoculation, on day 7, by the technique described by Lennettee[5].

The aqueous stock solution of berberine sulphate was autoclaved at 15 lb pressure per square inch for 15 minutes and stored at about 4°C. The concentration of berberine was so adjusted that the required dose for each egg was present in 0.2 ml volume; this was diluted with an equal volume of autoclaved sucrose potassium glutamate (SPG) medium[6]. Different doses (0.05, 0.2, 0.4, 0.5, 0.6 and 1.0 mg) of berberine were inoculated to study its direct effect on chick embryos.

The isolates "strain TRIC/India/A.I.I.M.S. - 160/0" and "strain TRIC/India/A.I.I.M.S. - 285/0") of trachoma organisms were obtained from trachoma patients and maintained by repeated passages through the embryonated eggs. For preparing the stock

suspension of these organisms, heavily infected yolk sac membrane was triturated with about 3-4 ml of SPG medium in a mortar and pestle and stored at -70°C. The suspension was thawed before use and its serial dilutions (10ß^{1}, 10ß^{2}, 10ß^{3}, 10ß^{4}, 10ß^{5}) were made in SPG medium. Doses of 0.2 ml of undiluted suspension and each of its dilutions were further mixed with 0.2 ml SPG medium and inoculated into 7 day old eggs to study their direct effect on the chick embryo.

For studying the effect of berberine on trachoma organisms, highest tolerated dose (0.5 mg/egg) or lower doses of berberine and the lethal doses of trachoma organisms (0.2 ml undiluted suspension and its 10ß^{1} dilution) were mixed, *in vitro*, and incubated for 30 minutes in an ice container prior to inoculation. For positive control, a known antitrachoma drug-sulphadiazine (1 mg/egg) was used[7]. For every egg, the final inoculation volume was 0.4 ml and each dose combination was inoculated into 4 eggs.

The inoculated eggs were incubated at 45°C and candled each day. Death of embryos within 2 days after inoculation was considered non-specific. Subsequent deaths were recorded and analysed. The embryos which survived for 11 days post-inoculation were deshelled on day 12. The yolk sac membranes of the dead or deshelled embryos were dissected, their smears made, stained with Gimenez stain and the presence of elementary bodies studied microscopically (X 100).

Studies on experimentally-induced trachoma in monkey eyes

Four healthy adult monkeys free from eye diseases were used for the study. The suspension of trachoma organisms was gently and thoroughly rubbed on the conjunctiva of both eyes of all the 4 monkeys with cotton swab wicks. If required, another application was made after 72 hours. After the conjunctivitis was clinically discernible (which took 2-3 days), berberine sulphate (0.2%) was instilled in both eyes three times a day in two monkeys; 20% sulphacetamide was used in the third monkey. The remaining was instilled with plain distilled water

and served as control. Drug instillations were continued till the eyes became normal after which the animals were observed for a period of 30 days to study the chance of relapse.

RESULTS

Experiments on *in vitro* antiheparin action

Normally, blood samples were found to coagulate in 1 to 4 minutes. Berberine in the doses of 0.3-3 mg/ml of blood (6 samples each) had no effect on the blood coagulating time. However, at 10 mg/ml dose, it exerted a direct anticoagulant action in that, it rendered 4 of the 6 samples incoagulable for 24 hours; fifth sample remained partially coagulated and the sixth showed normal coagulation. At this dose, protamine and toluidine blue also impaired blood coagulation.

50 I.U. heparin completely prevented coagulation of 1 ml blood or of its plasma over 24 hour observation period. In a dose of 0.3 mg berberine coagulated of the heparinized blood samples of dog, 1 mg coagulated 9 out of 12 samples and 3 mg dose coagulated all the 29 samples. The period between addition of berberine and formation of complete clot was inversely dependent on the dose of berberine; thus, 1 mg dose of berberine produced coagulation in an average time of 5 minutes and 3 mg took about 3 minutes. On the other hand, 10 mg dose of berberine (which itself exerted anticoagulant action) did not coagulate any of the 5 heparinized samples. The heparinized blood samples of man, goat and cattle (6 samples each) were coagulated equally effectively by 3 mg dose of berberine.

In 0.3 and 1 mg doses, protamine coagulated 5 out of 8 and 2 out of 6 heparinized samples respectively. Three and 10 mg doses of protamine failed to coagulate the heparinized blood. Toluidine blue did not clot any of the 8 samples at 0.3 mg dose but 1 and 3 mg doses coagulated 5 out of 7 and 5 out of 8 samples respectively; 10 mg dose (6 samples) failed to coagulate 4, induced partial coagulation in 1 and coagulated the remaining 1 sample.

Atropine, morphine, physostigmine, strychnine, quinidine and quinine failed to coagulate the heparinized dog blood samples over 22 hours, if 3 mg berberine was added to those samples after 22 hours, there was prompt and complete coagulation.

Berberine, protamine and toluidine blue, upto a dose of 10 mg, failed to coagulate the dog blood or plasma samples rendered incoagulable by Na_2 EDTA, potassium oxalate or sodium citrate.

Experiments on antitrachoma action in chick embryo

The control eggs inoculated only with 0.4 ml of SPG medium survived the entire 12 day period. Berberine in the dose range of 0.06 to 0.5 mg/egg did not affect the development of embryos; however, 0.6 and 1.0 mg/egg doses of berberine produced 50 and 100 per cent mortality respectively.

In a dose of 0.2 ml/egg, the undiluted suspension of trachoma organisms killed 75-100% embryos and their 10ß^{1} concentration produced 50-75% mortality. Lower concentrations of the suspension (10ß^{2} to 10ß^{5}) generally produced lesser mortality and took longer time to kill the embryos. Apparently, the two isolates of the trachoma organisms exerted more or less equal lethal effect. Irrespective of the dilution of the inoculum and incidence of mortality, yolk membrane of every inoculated embryo showed abundant trachoma elementary bodies.

The highest tolerated dose of berberine (0.5 mg/egg) was used for studying its antitrachoma action. This dose of berberine as also of sulphadiazine (1 mg/egg) definitely reduced the embryo mortality induced by the highly lethal doses of trachoma organisms suspension (Table 1). An equally interesting fact associated with the use of these 2 drugs was that the yolk sac membrane of protected and even unprotected embryos did not show elementary bodies (Table 1).

Studies on antitrachoma action in monkey eyes

Monkeys infected with trachoma organisms developed severe conjunctivitis within 96 hours. Instillation of the 0.2% solution of berberine caused the recovery within 15-18 days; 20% sulphacetamide cured it in 20 days. Hyperaemia of the eyes disappeared earlier in monkeys receiving berberine while disappearance of follicles was

quicker in sulpha-treated monkey. No apparent sign of irritation of the eyes was observed in the monkeys receiving berberine or sulphacetamide. In none of the animals receiving these drugs, relapse of trachoma was observed even after 30 days of recovery. However, the control monkey developed a typical trachomatous picture comprising of follicles at the upper tarsal conjunctiva, the scrapings from which showed inclusion bodies. The follicles remained visible even after 50 days of infection.

TABLE 1 - EFFECT OF BERBERINE ON CHICK EMBRYOS INFECTED WITH TRACHOMA ORGANISMS (ISOLATE "TRIC/India/A.I.I.M.S - 285/0)"**

Inocula	Dose/ Egg	No. of embryos found alive (or dead) on Days Post-inoculation												
		No. of Eggs used	1	2	3	4	5	6	7	8	9	10	11	12
Organisms (undiluted)	0.2 ml	4	4	4	4	1 (3) + + +	1	0 (1) + + +						
Organisms (undiluted) + Berberine	0.2 ml + 0.5mg	4	4	4	4	4	4	4	3 (1) -ve	3	3	3	3	3 -ve
Organisms (dilued, 10^{-1})	0.2 ml	4	4	4	4	4	4	4	4	3 (1) + + +	3	2 (1) + + +	2	2 + + +
Organisms (diluted, 10^{-1}) + Berberine	0.2 ml + 0.5mg	4	4	4	3 (1) -ve	3	3	3	3	3	3	3	3	3 -ve
Organisms (diluted, 10^{-1}) + Sulphadiazine	0.2 ml + 1.0mg	4	4	4	4	4	4	4	3 (1) -ve	3	3	3	2 (1) -ve	2 -ve

** Almost similar were the effects on Isolate "TRIC/INDIA/A.I.I.M.S. 160/0".
+ + + indicates the presence of elementary bodies and -ve indicates their absence.

DISCUSSION

Antiheparin action

In *in vitro* experiments, the effects and potency of berberine remarkably resembled those of the two standard antiheparin drugs, protamine sulphate and toluidine blue. These agents neutralize the anticoagulant effect of heparin in smaller doses; in larger doses, they act as anticoagulants. The mechanism(s) of direct anticoagulant effect of these compounds is (are) not yet known.

Heparin molecule is the strongest organic acid occuring within the body and its anticoagulant effect is partly due to this acidity[8]. Molecule of protamine is rich in unmasked electric charges and toluidine blue has three sites which are potentially capable of bearing a positive charge and both of which, therefore, can neutralize charges on heparin[9,10]. However, this phenomenon cannot explain the *in vitro* antiheparin action of berberine because berberine molecule does not possess any strong free electric charges[11] since it has only one possible site for a positive charge in the tautomeric form. Even this may be masked when berberine is dissolved in water. Furthermore, the site is vicinal to the double bond. Therefore, it is theoretically unlikely that berberine and heparin are forming electrostic complex. Further, the antiheparin action of berberine is not due to its alkaloidal nature since other alkaloids failed to coagulate the heparinized blood. Like protamine and toluidine blue, berberine did not coagulate the blood and plasma samples containing EDTA, potassium oxalate and sodium citrate. This suggests that their antiheparin action is not nonspecific.

Like protamine, berberine may be used for the *in vitro* assay of circulating heparin. However, further work is required to explore such a possibility.

Toluidine blue, unlike protamine, can be given orally in clinical practice and it has been claimed to benefit some cases of bleeding disorders including hypermenorrhoea[12,13]. It is interesting to note,

in this context, that crude preparations of plants containing berberine have been traditionally used in India[14] for conditions characterized by bleeding including hypermenorrhoea. On the other hand, there are conflicting clinical[14] and experimental[10] reports which indicate that these preparations may aggravate bleeding particularly at the site of application. It is possible that the antiheparin and direct anticoagulant actions of berberine at low and higher doses respectively might explain the conflicting reports.

Altogether, it is difficult to comment on the therapeutic role of berberine in the treatment of diverse bleeding disorders. The clinical evaluation of such drugs is neither easy nor a quick task. Nevertheless, a systematic clinical and experimental work needs to be carried out and till adequate studies are conducted, it would be wise not to disregard a tradition which is ancient, persistent and widespread.

Antitrachoma action

The present study indicates that 0.5 mg dose of berberine is as effective as 1 mg of sulphadiazine in reducing the mortality and infectivity of trachoma organisms. The 0.4 mg/egg dose of berberine causes reduction or even disappearance of elementary bodies from the yolk sac membrane but does not produce any gross teratogenic or lethal effect upto a dose of 0.5 mg/egg; which indicates that this dose is apparently nontoxic for developing embryos.

The antitrachoma activity of berberine is not due to the effect of its pH which is about 6.8-7.2 after autoclaving. This pH is quite optimal for the growth of trachoma organisms[7]. The possibility of berberine interfering with protein and nucleic acid synthesis by the trachoma organisms needs to be explored. Berberine is known to form a complex with DNA[15] and to inhibit the RNA and protein synthesis by micro-organisms[16,17].

The present findings of the experimental studies on berberine confirm its clinical efficacy in trachoma patients observed earlier[4]. Also, the observations on a limited number of monkeys indicate that berberine is effective in the form of aqueous eye drops and cures the trachoma by simple instillation. It also appears that, on weight basis, berberine is about 100 times more effective than sulphacetamide in monkeys. Berberine, therefore, may prove a practical remedy for large-scale use in trachoma patients. However, the encouraging results, both on chick embryos and on monkeys eyes, should first be confirmed carefully in clinical trials. The issues like its prophylactic use, rate of cure at different stages, synergism with other drugs, rate of reinfection or relapse and duration of treatment will require cautious clinical attention of the ophthalmologists.

Berberine has also been shown to possess antibacterial[16,18] antifungal, local anaesthetic, antihistamine[19] and anti-inflammatory[20] actions. It is likely that such complementary actions may further contribute to its clinical efficacy in eye diseases.

These actions of berberine strongly support the clinical efficacy of *Berberis aristata* in bleeding disorders and in eye diseases as claimed by Bu-Ali-Sina[1] and Nafisi[2].

SUMMARY AND CONCLUSIONS

In 1-3 mg doses, berberine neutralized, *in vitro*, the anticoagulant action of 50 I.U. heparin per ml of blood or its plasma. The antiheparin potency of berberine was comparable, or close, to those of protamine sulphate and toluidine blue. Neither berberine nor protamine or toluidine blue induced coagulation in the blood samples rendered incoagulable by EDTA, potassium oxalate or sodium citrate. Like protamine sulphate and toluidine blue, larger dose (10 mg/ml) of berberine exerted a direct anticoagulant effect. The mechanism of this paradoxical phenomenon is not known.

In a dose of 0.5 mg/egg, berberine protected 50-75% chick embryos from the lethal effect of trachoma organisms; also, it completely inhibited the development of infective elementary bodies in the yolk sac membrane. One mg/egg dose of sulphadiazine was equally effective. Although 0.4 mg/egg dose of berberine failed to protect the embryo, it considerably reduced, or completely inhibited the number of elementary bodies.

Daily instillation of 0.2% aqueous solution of berberine clinically cured the experimentally-induced trachoma in monkey eyes within 15-18 days. Suphacetamide (20%) treatment was equally effective.

As this work supports the early clinical observations of Bu-Ali-Sina[1] and Nafisi[2] about the usefulness of berberine containing plant *Berberis aristata* in bleeding disorders and in eye diseases, adequate clinical trials are required to assess the utility of berberine in such affections.

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TRUFFLES IN EYE DISEASE

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It is mentioned that the use of truffles increased in the days of the Prophet (ﷺ). Earlier, people refrained from eating it stating that it is the smallpox of the earth. When the Prophet (ﷺ) heard about this, he said:

> "*Truffles are like manna (i.e. they grow naturally without man's care) and their water heals eye diseases*".

Ibn Sina mentioned that its water was boiled then cooled and used as Kohl (painting of the palpebral conjunctivae).

Truffles are a type of fungus that grow about 30 cms, below the surface of the ground, in groups of10-20 lumps. Each lump is circular or oval, few centimetres in diameter with a smooth surface and soft consistency. Its colour ranges from brown to grey to black. It grows in Arabia, Syria, Jordan and Palestine. In Europe, it is grown mostly in France and Italy.

Truffles or *Terfeziz claveyi* are a division of Ascomycetae, constituting numerous spore containing scars. There are three types of Truffles growing in Kuwait, their classification has been carried out by the native beduins according to the colour and size, namely - Zobaidy, Khollasy and Rajaye. Analysis carried out by El-Gindy and Alami in Kuwait showed no significant difference in the constituents of the three types.

Arabs sometimes call it the plant of thunder because it grows after rainy seasons accompanied with thunder. Having no root,

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* Bulletin of Islamic Medicine, 1: 353 - 357, 1981.

stem, leaves or flowers, it does not belong to the plant kingdom. It grows spontaneously without any effort of cultivation. Even trials to cultivate it has failed. This supports the Prophet's (ﷺ) saying that it is like manna i.e. gift from God to people.

MATERIAL AND METHOD

Truffles imported from Kuwait were carried to Odessa during our visit to the Soviet Union. The aquous extract was prepared in Filatov Laboratory by the method of extraction named according to Filatov.

The extract was dried in the Central Serogic Laboratories of the Ministry of Puplic Health in Cairo. The powdered extract was kept to be dissolved in distilled water just before use. The resulting solution will be of the same concentration as that of natural truffles water. This water is brown in colour with a characteristic pungent smell.

The following trials were carried out using truffles water.

A. Bacteriological Action

Cultures were prepared of different gram positive and gram negative bacteria. Truffles water was applied to the culture. It showed no bacteriostatic or bactericidal activities.

B. Trials with Truffles water in cases of cataract

59 cases of cataract were selected. They included cases of soft, hard and complicated cataracts.

Truffles water was used in the form of eye drops five times daily for three years. The trail showed no effect of truffles on the course of cataract. Conjunctival congestion, occasionally severe, appeared in some cases.

C. Trials with Truffles water in cases of trachoma

600 children in madrassas teaching the Holy Quran were examined. Trachoma in its three stages was diagnosed in 86 cases. These were treated for one month according to the following regimen.

First Clinical Trial

It included 30 children suffering from early first stage of trachoma where the lymphoid follicles can be detected in the conjunctival mucosa only by magnification. The cases were divided into two groups each comprising 15 children. The two groups were homogenous from the clinical point of view i.e. the distribution of the trachoma follicles on the conjunctivae was nearly equal in both groups.

1 - The first sub group (experiment 1) was treated by chloramphenicol eye drops 5 times daily. Before bed time tetracycline ophthalmic ointment was applied.
2 - The second sub group (experiment 2) was treated with chloramphenicol drops 5 times daily + tetracyline ointment before bed time + truffles water 5 times per day.

Second Clinical Trial

This comprised 40 children suffering from active trachoma where the lymphoid follicles could be detected by the naked eye. Among them were 12 cases suffering from corneal pterygium.

This group was futher sub-divided into 4 sub-groups each comprising 10 cases (of which 3 had pterygium). Treatment was carried as follows:

1 - First sub-group (experiment 3) included 10 cases treated by chloramphenicol eye drops 5 times daily + tetracycline ointment before bed time.
2 - Second sub-group (experiment 4) included ten cases treated as above + truffles water 5 times daily.
3 - Third sub-group (experiment 5): After extraction of the content of the lymphoid follicles they ware treated by chloramphenicol eye drops 5 times daily and tetracycline ointment at night.
4 - Fourth sub-group (experiment 6): Same as previous group (experiment 5) + truffles water 5 times daily.

Third Clinical Trial:

Included 16 cases suffering from active trachoma together with marked follicular conjunctivitis. They were further subdivided into two sub-groups:

1 - First sub-group (experimet 7) where 8 cases were treated with dexamethasone eye drops 5 times daily + steroid ophthalmic ointment before bed time.
2 - Second sub-group (experiment 8) where 8 cases were treated with the same regimen as experiment 7 together with truffles water 5 times daily.

RESULTS

From the table we can observe the effect of combination of truffles water to the traditional treatment of trachoma in its various stages.

Microscopic examination of conjunctival scrapings was carried in 4 cases (2 from experiment 5 where antibiotics were used after follicular extraction) and (2 from experiment 6 where truffles water was added). Microscopy revealed marked lack of lymphocytes and scanty fibrous tissue in experiment 6 where truffles warter was used. This was in contrast to experiment 5 where cases showed abundant lymphocytic infiltration and fibrous tissue formation.

DISCUSSION

Trachoma is a chronic contagious disease of the Mediterranian area and other parts of the world particularly Japan. Its complications were responsible for 25% of cases of blindness in endemic areas before the era of antibiotics. Trachoma is caused by a virus affecting the conjunctival cells lining the eye lids and covering the globe. In its early stages it causes conjunctival congestion, and marked infiltration by lymphocytes that collect under the conjunctival lining to form small lymphoid follicles. Their size is 1-2 mm and can be seen by the hand lens. They may heal spontaneously, by the

appearance of fibroblasts replacing these follicles, or the disease may progress to the second stage where lymphocytic infiltration increases forming large lymphoid follicles under the lining, conjunctival layer-capillary changes appear around the follicles surrounding them together with fibroblasts. In the centre of the follicle may be one or more macrophages engulfing small lymphocytes. At that stage the follicle appears protruding with its yellowish colour on the conjunctival surface. At that stage, it can be extruded by a special forcep. The virus activity may continue leading to abnormal growth of conjunctival lining cells much more faster than the degree of vascularisation. This leads to atrophy of some superficial cells. Around these follicles the process of fibrosis starts. This marks the onset of the stage of regression which ends by contraction of the fibrous tissue leading to entropion and rubbing lashes. On the contrary, the lymphocytic infiltration may spread reaching the cartilage which becomes weak; thus the eye lid loses the power to retract completely.

Other complications include corneal congestion, vascularisation and pterygium formation; or obstruction of the lacrimal duct. The friction of the rough conjunctiva with the cornea leads to corneal opacities that affect vision. Superadded infection may complicate the condition or angular conjunctivitis may be superadded.

Treatment leads to fibrous tissue formation in place of damaged lymphocytes and conjunvtival cells.

The severity of complications depends on the extent of fibrosis.

The significant effect of truffles water in the above mentioned clinical trial is that it minimises the degree of fibrosis at the site of the injury.

CONCLUSION

From the above we can conclude that truffles water hinders the process of fibrosis in cases of trachoma. This is achieved by interference with fibrocyte formation. Such an effect may be ascribed to the neutralisation of the toxins liberated from the virus and interference with cellular infiltration. At the same time, it prevents the abnormal growth of conjunctival cells and increases their nutrition by promoting vascularisation.

Considering that most of the complications of trachoma are the result of fibrosis we can conclude that truffles water prevents most of the complications of trachoma. The therapeutic effect of truffles was mentioned by the Prophet (ﷺ) 14 centuries ago when no facilities for scientific investigation were available.

TABLE SHOWING RESULTS OF TREATMENT OF TRACHOMA BY TRADITIONAL TREATMENT WITH OR WITHOUT TRUFFLES WATER.

No. Of Experiment	No. Of Cases	Treatment	Results
1	15	Chloramphenicol drops + tetracycline ointment	Cure of trachoma-fibrosis of conjunctiva in 8 cases
2	15	Chloramphenicol drops + tetracycline ointment + truffles water	Cure of trachoma - conjunctiva returned to normal except one case of fibrosis.
3	10	Chlorapmphenicol drops + tetracycline ointment	Follicles showed no change.
4	10	Chlorapmphenicol drops + tetracycline ointment + truffles water	Follicles showed no change, capillary growth in conjunctiva.
5	10	Follicle extrusion + Chloramphenical + tetracycline	Conjunctiva healed with fibrosis in all cases. No definite pattern to conjunctival capillaries. Pterygium healed.
6	10	Follicle extrusion + Chloramphenical + tetracycline + truffles water	Conjunctiva heald- capillary growth in conjunctiva in 7 cases, fibrosis in 3 cases, pterygium heald.
7	8	Steroid drops and ointment	Cases cured. Healing with some fibrosis in conjunctiva.
8	8	Steroid drops and ointment + truffles water	Cases cured, residual conjunctival congestion- no fibrosis.

PROTECTIVE EFFECT OF GUL-E-TEESU (BUTEA MONOSPERMA FLOWERS) IN EXPERIMENTAL LIVER INJURY

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PROTECTIVE EFFECT OF GUL-E-TEESU (BUTEA MONOSPERMA FLOWERS) IN EXPERIMENTAL LIVER INJURY*

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Abstract

An aqueous extract of Gul-e-teesu (flowers of *Butea monosperma*) offered protection against experimentally induced liver injury by CCl_4 in albino rats as shown by biochemical and histopathological studies. In pentobarbitone sleeping time studies the extract showed improvement of the metabolic functions in these liver injured animals. In partial hepatectomised rats it showed significant increase in rate of liver regeneration.

INTRODUCTION

When Arab medicine was introduced in India they incorporated known and new Indian drugs and added to the materia medica of Islamic system of medicine. Gul-e-Teesu (flowers of *Butea monosperma* syn.*B.frondosa*) belonging to the family Leguminaceae was one of them[1]. The flowers are claimed to be tonic, aphrodisiac, diuretic and yield yellow dye[3,4]. It was also claimed to be a blood purifier, anti-inflammatory, corrective of humours namely bile, phlegm and black bile[5], anthelmintic, antipyretic, appetizer and used in splenomegaly[6] and viral hepatitis[7].

A crystalline fraction composed of the glycosides butrin and plasitirin isolated from the alcoholic extract of the petals reduced

* Bulletin of Islamic Medicine, 1: 448-453, 1981.

the number of implants in the mated rats[8]. A good deal of controversy exists regarding the antioestrogenic effect of the alcoholic extract of the petals of the flowers. The present study communicates the experimental investigations conducted to evaluate the effects of Gul-e-teesu (B.M.) on carbon tetrachloride induced liver injury and on partial hepatectomised experimental animals.

MATERIALS AND METHODS

Flowers of B.M. were collected and an aqueous extract was prepared by keeping the flowers (2 gm) in boiled distilled water (24 ml) and the supernatant was decanted after 16 hours and used for the experiments. Albino rats of either sex weighing 130 to 150 gm were divided into 4 groups of six animals each. Group I served as control and was given distilled water (2 ml/100 gm) for all the 8 days. Group II animals were given carbon tetrachloride at the dose level of 0.2 ml/100 gm subcutaneously for 3 consecutive days. Group III animals were pretreated for 5 days with the drug BM (333 mg/150 gm) orally and also given along with CCl_4 for next 3 days. Group IV animals were treated with B.M. for all the 8 days.

Effect on Pentobarbitone Sleeping Time: On the 8th day, liver functions and the extent of liver damage were assessed by estimating the pentobarbitone sleeping time in all the animals according to the method of Bhide[9].

Biochemical Studies: On the 9th day blood samples were collected in sterile test tubes for biochemical estimation of serum bilirubin[10] serum alkaline phosphatase[11], SGPT[12], total protein (Folin Lowrey Method[13]), albumin and globulin and A/G ratio[14].

Histopathological Studies: On the 9th day, all the groups of animals were sacrificed and the liver lobes were removed from identical site of each lobe and preserved in 10% formal saline for 24 hours. Paraffin sections (5 micron thick) were prepared and stained

with haemotoxyline and eosin and studied at 100x and 225x magnifications. The criteria used for histological assessment of liver injury were:

(1) Extent of hydrophic degeneration

(2) Extent of fatty changes

(3) Extent of inflammatory / leucocytic infiltration

(4) Hepatic cellular necrosis

(5) Overall assessment.

Effect on Liver Regeneration on Partial Hepatectomised Rats:

This was studied in 70 male Wistar Albino rats ranging from 150 to 220 gm and divided into 10 groups of 7 animals each. Partial hepatectomy was done in all animals according to the method of Brues *et al*[13]. The drug B.M. was administered orally (333 mg/100 gm) in the test group daily and distilled water (2 ml) for the control group. The animals were sacrificed after the first (24 hours), 2nd, 3rd, 5th and 7th day and the livers were removed, dried between two filter papers and weighed. The liver body weight index was calculated and the effects on treated and untreated animals were compared as reported by Kameswaran and Nazimuddin[16].

RESULTS

Effect on Pentobarbitone Sleeping Time: In group II, treated with CCl_4 alone, the pentobarbitone sleeping time was considerably more than the controls due to the extensive liver damage and the inability of the injured liver to metabolise pentobarbitone. In group III (CCl_4 + B.M.), the pentabarbitone sleeping time was almost comparable to that of normal control, showing the improved liver function. Group IV (B.M. alone), did not have any significant effect on pentobarbitone sleeping time. The results are presented in table I.

Biochemical Changes

Plasma Proteins: Estimation of plasma proteins in the various groups of animal revealed the following. It was observed that there was no significant change in the total proteins between all the four groups. While the A/G ratio in the normal control rats was found to be 1:0.69 in animals treated with CCl_4 alone (Group II) the A/G ratio was found to be 1:2.12 thereby showing a significant reversal ($P < 0.001$), while there was no change in the A/G ratio of animal treated with B.M. alone (Group IV). There was a definite indication of inhibition of A/G ratio reversal in animals treated with CCl_4 and B.M. (Group III). Administration of B.M. definitely appears to have an inhibitory activity in the reversal of albumin globulin ratio induced by CCl_4 ($P < 0.01$). The results are presented in table II.

Serum Bilirubin: It can be seen from table II that there was no significant change in the levels of serum bilirubin between the normal control (Group I) and B.M. treated animals (Group IV). However, in animals treated with CCl_4 alone (Group II) there was a steep increase in the levels of serum bilirubin ($P < 0.001$) and administration of B.M. to animals treated with CCl_4 (Group III) appear to inhibit the elevation of serum bilirubin which was found significant ($P < 0.01$).

Serum Alkaline Phosphatase: Here again, it can be seen from table II that there was no significant difference in the serum alkaline phosphatase activity between the normal control (Group I) and B.M. treated (Group IV) animals while there was a significant elevation in the alkaline phosphatase activity in the CCl_4 treated animals (Group II) compared to Group III animals ($P < 0.001$). It was observed that B.M. has a definite role in preventing the increase of alkaline phosphatase activity induced by CCl_4.

SGPT: There was no difference between group I and Group IV animals. However, in animals treated with CCl_4 (Group II) SGPT values show a tremendous increase ($P < 0.001$) and in animals

treated with CCl_4 + B.M. (Group III) such a significant increase was not observed. The results are presented in table II.

TABLE I
EFFECT OF DIFFERENT TREATMENTS ON PENTOBARBITIONE SLEEPING TIME IN RATS

Group	Sleeping Time in Minutes		
	Mean	SD	P. Value
I Control	65.5	8.5	-
II CCl_4	150	15.5	< 0.001
III CCl_4 + BM	80.5	10.5	N.S.*
IV B.M.	70.5	8.0	N.S.*

*N.S. = Not significant with respect to controls

TABLE II
EFFECT OF DIFFERENT TREATMENTS ON BIOCHEICAL PROFILES IN RATS

Groups	Total Protein gm% M + SD	Albumin gm% M+SD	Globulin gm% M + SD	AG Ratio gm% M + SD	S. Bilirubin M + SD	S. Alk. PO4 K + A Unit M + SD	SGPT i.u. M + SD
I	6.72±0.16	3.83±0.20	2.66±0.39	1:0.69	0.27±0.02	3.47±0.41	5.17±0.30
II	6.28*±0.20	1.98***±0.34	4.19***±0.34	1:2.12***	0.95***±0.04	133***±4.08	46.83***±3.13
III	6.43*±0.27	3.31+ ±0.12	3.12*±0.23	1:0.94**	0.53**±0.04	55.26**±3.44	17.0+ ±2.83
IV	6.66*±0.24	3.90*±0.20	2.20±0.25	1:0.56*	0.32+ ±0.02	4.05*±0.50	6.55*±0.40

M = Mean; SD = Standard Deviation
*P = N.S. (not significant); **P < 0.01; ***P < 0.001; +P < 0.05

TABLE III

EFFECT OF BUTEA MONOSPERMA FLOWERS (B.M) ON LIVER REGENERATION IN PARTIALLY HEPATECTOMISED RATS

Day	Control				B.M.			
	Body Wt.	Expected Liv. Wt.	Actual Liv. Wt.	Liv. Reg. %	Body Wt.	Expected Liv. Wt.	Actual Liv. Wt.	Liv. Reg. %
1.	165.83	5.86	6.08	103.75	154	5.50	6.83	124.18*
2.	156.33	5.58	6.48	116.13	151	5.43	10.23	188.4*
3.	176.85	6.19	8.13	131.34	160	5.69	10.76	189.1*
4.	211.25	7.72	8.29	114.82	163	5.78	10.53	182.18*’
5.	172.14	5.41	8.74	161.55	165	5.41	11.24	207.76*

*$P < 0.01$

Histopathological Studies

In CCl_4 treated animals (Group II) a swelling and hydrophic degeneration of the contralobular hepatic cells developed. These changes progressed to a diffuse fatty degeneration and midzonal necrosis followed by the leucocytic infiltration. The necrosis in some cases was also focal. The intermediary zone presented a number of baloon cells. But in CCl_4 and B.M. treated animals (Group III) hepatic cell walls were normal. Hepatic cells did not show fatty infiltration / degeneration in almost all lobules and there were no nuclear changes in liver cells. There was no bile stagnation seen in the biliary cannaliculi and the normal architecture was well preserved. Only a mild infiltration of round cells were seen in portal tract. They did not have any deleterious effect on the liver. The difference between the sections of the control group (Group I) and B.M. treated (Group III) were minimal characterised by absence of nuclear pathology, inflammatory infiltration, pigmented disturbance and fibrosis. Thus the drug appears to have a definite protective effect by way of preventing the deleterious effect of CCl_4 on liver.

Effect on Partially Hepatectomised Rats

It can be seen from Table III that the liver regeneration in B.M. treated animals is much more higher than the controls and statistically significant. While the regeneration of liver in the control animals was 103.75%, 24 hours after partial hepatactomy and 161.55% after 5 days, in animals treated with B.M. the percentage regeneration of liver in the 24 hours study and 5th day study was 124.18% and 207.76% respectively which have been found to be significant ($P < 0.01$).

Discussion

The results of the present study reveal that the aqueous extract of the flowers of B.M. has a definite protective effect against the deleterious effect of CCl_4 upon the structure and function of liver as estimated by various parameters. The pentobarbitone sleeping time measurement which is an important parameter in assessing the liver function clearly shows that in animals treated concurrently with CCl_4 and B.M. the duration of sleeping time is almost similar to that of normal control groups, thereby confirming the ability of aqueous extract of B.M. to improve the metabolic function of the liver. The biochemical studies also seem to support the beneficial effect of the B.M. in antoganizing the toxic effect of CCl_4 on liver. While there is no significant change in the levels of total proteins, A/G ratio, serum bilirubin, serum alkaline phosphatase and SGPT between the normal controls and the animals treated with B.M. alone, the CCl_4 treated animals show a significant reversal in A/G ratio and a sharp increase in the levels of serum bilirubin, serum alkaline phosphatase and SGPT. The above CCl_4 induced biochemical changes are effectively antogonised by concurrent administration of B.M. which again confirms the protective ability of B.M. against the hepatotoxic effect of CCl_4.

Apart from this the histopathological studies also confirm the beneficial role of the B.M. in antagonising the deleterious effect of

CCl_4 on the histology of the liver. While the CCl_4 treated animals show extensive histological changes, the animals concurrently treated with CCl_4 and B.M. show only a mild to moderate histopathological changes. Effect on partially hepatectomised rats also indicate that B.M. is able to accelerate the process of regeneration of liver and this is one positive indication of the beneficial effect of B.M. Further studies are being carried out to determine the effect of B.M. on mitotic index in partially hepatectomised rats which would give a clear picture as to the effect of the test drug on proliferations of hepatic cells after partial hapatectomy.

Since, the exact mechanism of CCl_4 induced hepatotoxicity is not known[17], it is not possible at present to postulate any precise mode of action by which B.M. confers protection against CCl_4 induced hepatitis. The findings of the present study strongly support the claims of Unani Physicians regarding the use of these flowers in the treatment of hepatic disorders. Further, studies are required to identify the active principles in the aqueous extract of *Butea monosperma* and also to elucidate the precise mechanism by which the extract is able to protect the liver from the toxic effect of CCl_4 and accelerate the regeneration of hapatic cells.

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ANTI-INFLAMMATORY EFFECT OF GUL-E-TESU (BUTEA MONOSPERMA FLOWERS)

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ANTI-INFLAMMATORY EFFECT OF GUL-E-TESU (BUTEA MONOSPERMA FLOWERS)*

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INTRODUCTION

Gul-e-teesu (*Butea Monosperma*, Syn. *B. frondosa* flowers) belong to the family Leguminaceae. The flowers are claimed to be tonic, aphrodisiac, diuretic and yield yellow dye (Chopra *et al*[1], Nadkarni[2], Satyavathi *et al*[3]). It was also claimed to be a blood purifier, anti-inflammatory, corrective of humours namely bile, phlegm and black bile (Mohd. Hussain[4]), anti-helminthic, antipyretic, appetiser and used in splenomegaly (Najmul Ghani Khan)[5]. A crystalline fraction composed of the glycosides butrin and plasitirin isolated from the alcoholic extract of the petals reduced the number of implants in the mated rats (Kapila *et al*)[6]. A good deal of controversy exists regarding the antioestrogenic effect of the alcoholic extract of the petals of the flowers. An aqueous extract of the flowers was reported to posses a protective effect in experimental liver injury (Nazimuddin *et al*)[7] and effective against viral hepatitis (Shakira *et al*)[8]. The present study deals with the findings of the Unani drug, Gu-e-Teesu (*Butea monosperma*) on the activity against experimental inflammation.

The anti-inflammatory activity of *Butea monosperma* flowers (B.M.) was evaluated by (I) rat hind paw oedema, (II) cotton pellet granulation tissue formation and (III) granuloma pouch methods, the first method being an acute inflammatory model (exudative) and the latter two being chronic inflammatory models (proliferative).

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MATERIALS AND METHODS

Acute Inflammation

It was induced by rat hind paw oedema method (Winter *et al*)[9], in three groups of rats (5 animals in each). Oedema was induced by injecting 0.1 ml of 1% carrageenin, 30 min after administering the following: Group I, control-distilled water (2ml/150gm); Group II, B.M. (2ml/150 gm) and Group III phenylbutazone (100 mg/kg). The volume of each paw was measured plethysmographically before and 4 hours after the injection of carrageenin. The percent inhibition of the oedema was calculated by the formula (1-T/C) x 100, where T and C are the mean paw volumes of drug-treated and control groups respectively.

Chronic Inflammation

a) *Granuloma Pouch:* This type of chronic inflammation was induced in 4 groups (5 in each) of rats as per the method described by Selys.[10] Granuloma pouch was induced in rats by injecting 20ml of air, followed by 0.5 ml of 2% v/v croton oil in ground nut oil, into the loose connective tissue between the shoulder blades. All the three groups of animals were administered with the following, 24 hrs before induction of granuloma pouch and subsequently continued for 7 days. Group I Control-distilled water 2ml/150gm, Group II Test-B.M. 2ml/150gm and Group III Positive Control - phenylbutazone - 100 mg/kg. All the animals were sacrificed on the 8th day, the pouch was opened and the exudate was collected and measured. The per cent inhibition of the volume was calculated by the formula previously mentioned.

b) *Cotton Pellet Implantation:* This was done according to the method of Winter *et al*[11], in 4 groups (5 in each) of rats by implanting sterile cotton pellets (10mg) subcutaneously in the groin region after anaesthetising the animals with ether. Administration of drugs was started 24 hrs before the implantation of the cotton

pellets and subsequently continued for seven days. The animals were administered with the following: Group I, distilled water 2ml/150gm., Group II B.M. 2ml/150gm and Group III phenylbutazone 100mg/kg. All the animals were sacrificed on the 8th day and the cotton pellets with the surrounding granulomatous tissue were removed, dried at 55°C for 24 hrs and the per cent inhibitions in the weight of the granuloma formation was calculated by the formula previously given.

Effect on total serum proteins and Albumin: Globulin ratio: Since there is a change in the albumin globulin ratio in experimental animals in chronic phase of inflamamtion (Werner)[12], blood was collected from the animals, in which inflammation was induced according to the method of Winter *et al*[11] and the total protein, albumin and globulin were estimated by the Biuret method[14].

Effect on adrenalectomised rats: Drugs which exhibited an anti-inflammatory effect in adrenal intact animals were studied for their effect in bilaterally adrenalectomised rats, in order to find out the involvement of adrenal glands in the mediation of their anti-inflammatory effect. Bilateral adrenalectomy was done according to Zarron *et al*[15] and the anti-inflammatory effect of the test compounds at a dose level of 2 ml/150gm of B.M. was studied according to the method of Winter *et al*[11].

Effect on rat peritoneal mast cells, *in vitro*: Lewis and Whitle[16] have reported that nonsteroidal anti-inflammatory drugs like indomethacin, flufenamate and meclofenamate stabilise the peritoneal mast cells of rat and prevent the rupture of mast cells and the associated histamine release, induced by pharmacological agents such as compound 48/80, and antigenic challenge. Hence an attempt has been made to elucidate the effect of B.M. on the rupture of mast cells induced by the mast cell degranulators such as

Compound 48/80, polymyxin-B, diazoxide and Triton X-100. Staining and counting of the ruptured and intact mast cells were done according to the technique described by Bray and Van Ansdel[17].

RESULTS

Anti-inflammatory effect: B.M. produced significant anti-inflammatory effects in rats as tested by the carrageenin induced hind paw oedema method, cotton pellet granuloma and granuloma pouch techniques. Table I summarizes these results.

Estimation of total serum proteins; Albumin: Globulin ratio

Table II summarizes the estimation of total serum proteins and albumin: globulin ratio. The data clearly show that B.M. has a definite role in preventing the albumin: globulin ratio reversal which occurs during inflammatory conditions.

Anti-inflammatory effect in adrenalectomised rats

Table III shows the anti-inflammatory effect in bilaterally adrenalectomised rats.

Effect on rat peritoneal mast cell *in vitro*

B.M. did not show any significant mast cell membrane stabilising effect, as evidenced by its inability to prevent the rupture of mast cell induced by Polymyxin-B, diazoxide, Triton X-100 and Compound 48/80.

DISCUSSION

B.M. has been found to produce significant anti-inflammatory effects in adrenal intact and bilaterally adrenalectomised rats. The exact mechanism by which it produces anti-inflammatory activity is not known. However, the present study has shown that there was no involvement of adrenal glands in the mediation of its anti-inflammatory effect and it did not stabilise the mast cell degranulating effect of various pharmacological agents such as compound 48/80 etc..., as a number of non-steroidal anti-inflammatory agents do.

Whether the anti-inflammatory activity of B.M. could be attributed to blocking postaglandin synthetase, needs further study. A number of anti-inflammatory agents such as steroids, aspirin like compounds, gold salts and pharmacological doses of estrogen have been shown to interfere with the immunological events, such as adjuvant - induced arthritis. In view of this, it would be of interest to extend such studies on B.M. as an immunosuppressive agent and to elucidate its role in various immunological and inflammatory reactions.

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TABLE I

ANTI-INFLAMMATORY ACTIVITY OF *BUTEA MONOSPERMA* FLOWERS

Group	Hind Paw Oedema Volume (ml)			Cotton Pellet Granuloma wt. of cotton pellet in mg.			Granuloma Pouch Volume of exudate in ml.		
	Mean ±SEM	% Reduction	P	Mean ±SEM	% Reduction	P	Mean ±SEM	% Reduction	P
Control	1.40 ±0.05	-	-	51.2 ±1.87	-	-	2.51 ±0.13	-	-
Phenylbutazone	0.39 ±0.03	72.20	< 0.001	23.7 ±1.37	53.70	< 0.001	0.78 ±0.08	72.20	< 0.001
Butea monosperma	0.60 ±0.05	57.15	< 0.01	30.2 ±1.64	41.00	< 0.01	1.19 ±0.08	52.60	< 0.01

TABLE - II

EFFECT OF *BUTEA MONOSPERMA* FLOWERS ON TOTAL PROTEIN (SERUM) AND A.G. RATIO

Group	Total Serum Protein g/100 ml.	Albumin g/100 ml.	Globulin g/100 ml.	A / G Ratio
Normal	4.293 ±0.03	0.805 ±0.03	3.488 ±0.04	0.230
Control	5.644 ±0.56	1.494 ±0.222	4.150 ±0.346	0.360
Phenyl butazone	4.270 ±0.12	0.845 ±0.045	3.425 ±0.20	0.246
B. monoperma	4.605 ±0.06	0.895 ±0.03	3.610 ±0.07	0.275

TABLE III

EFFECT OF *BUTEA MONOSPERMA* FLOWERS IN ADRENALECTOMISED RATS

Group	Cotton Pellet Granuloma wt. of cotton pellet in mg.			
	Mean	± SEM	per cent Reduction	P
Control	60.1	2.72	-	-
Phenylbutazone	30.1	1.19	49.92	< 0.001
B. monosperma	36.9	2.95	38.70	< 0.01

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A DOUBLE BLIND TRIAL OF MASTIC (SALADIN) AND PLACEBO IN TREATMENT OF DUODENAL ULCER

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A DOUBLE BLIND TRIAL OF MASTIC (SALADIN) AND PLACEBO IN TREATMENT OF DUODENAL ULCER*

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INTRODUCTION

Mastic is used for hundreds of years by Traditional Healers (*Attareen*) for relief of upper abdominal pain and acidity in many parts of Mediterranean region. The group of workers had a 65 years old female patient who was suffering from both benign gastric and duodenal ulcers which did not respond to currently used anti-ulcer drugs, but she responded dramatically, to oral Mastic, which was prescribed for her by a local Traditional Healer, in both relief of symptoms and healing of her ulcers.

The history of Mastic is lost in antiquity but both Pliny and Theophrastus mentioned it and the employment of Mastic in medicine dates back to the thirteenth century (Claus *et al*, 1970).

Ibn Al-Jazzar (died in 980 A.D.) the great Arab physician reported it for treatment of stomach ulcers. Also, Ibn Al-Baytar (died in 1248 A.D.) the other famous Arab physician mentioned it in treatment of intestinal ulcers (Materia Medica).

Mastic had been used since a long time by oriental women as Masticatory (British Pharmaceutical Codex). Mastic had been used by oriental women as Breath sweetner (Claus *et al*, 1970). The oil of Mastic is used by the Arabs for food and lights (Baily, 1935). Mastic

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is also used as a part of food, in many parts of Mediterranean region, such as in sweets and drinks (Tanker and Tanker, 1976). Mastic is a common article in oriental bazaars (Claus *et al*, 1970). In our country, Mastic is used in a spiritous drink called *Arakk Al-Mustakki*. In many parts of Turkey and Iraq particularly in Ninevah, Mastic is used as a part of many diets and sweets.

The resin of Mastic by itself or in a spiritous solution is used in Dentistry as a filling for carious teeth (Wren, 1971; British Pharmaceutical Codex 1949; Martindale Extra Pharmacopoeia, 1978). The Mastic paint (Pigmentum Mastiche Compositum) is used as a surgical varnish as protective covering for wounds to hold radium needles in position (Martindale Extra Pharmacopoeia, 1978). So far no side effects had been mentioned from use of Mastic in the last edition of Martindale Extra-Pharmacopoeia (1983).

Because of high incidence of active duodenal ulcer found in Arbil area among patients with dyspepsia subjected to Upper G.I.T. endoscopy (Al-Habbal and Huwez, 1982) and because of failure of some patients with duodenal ulcer to respond to the currently used anti-ulcer drugs and/or development of side effects to those drugs, the group of the workers decided to conduct a double blind control clinical trial on Mastic against placebo in treatment of duodenal ulcer in Arbil Teaching Hospital.

PATIENTS AND METHODS

Sixty patients with endoscopically proved duodenal ulcers entered the clinical trial. They were divided into two almost equal and well matched groups regarding age, sex and severity of duodenal ulcer; one group as a test group was given Mastic and the second group was given Lactose as placebo. Duodenoscopy (one forward viewing) was used for assessment of ulcer healing as duodenoscoy is the only satisfactory method for assessment of ulcer healing (Editorial, BMJ, 1980).

Both groups were studied as follows:

1. Nature of the procedure was explained to patients and their consents were taken.
2. Pregnant and lactating women were excluded.
3. Patients below 20 years of age were excluded.
4. Patients with pyloric stenosis were excluded.
5. All patients were advised to stop smoking and to avoid fried food, and aspirin.
6. All the drugs which promote ulcer healing were stopped. Patients with history of anti-ulcer drugs less than one month duration before the trial were excluded. Antacids (Gastrigel or Sinador tablets) were allowed to be taken on demand and daily demand were recorded.
7. Mastic or placebo were given in single daily dose (1gm) before breakfast for two weeks.
8. Both patients and Endoscopist were blind regarding the treatment.
9. At the end of the treatment, clinical evaluation and follow up duodenoscopy were done by the same Endoscopist. Ulcer healing was reported when the site of the original ulcer was completely replaced by epithelial tissue without appearance of other new ulcers (Chalabi, 1979).

RESULTS AND DISCUSSIONS

Sixty patients with endoscopically proved duodenal ulcers entered the clinical trial; 22 patients did not attend the follow up duodenoscopy and they were excluded. 38 patients (66%) completed the clinical trial and the number of the cases with their sex and age distribution of the two groups are shown in Table I. The results of the clinical trial are as follows:

1. Out of the 20 patients on Mastic who completed the trial 16 patients (80%) had complete symptomatic relief while 14 patients (70%) had complete endoscopic healing (Tables, 2 and 3).
2. In the control group, 18 patients completed the trial and only 9 patients (50%) had complete symptomatic relief while 4 patients (22.3%) had complete endoscopic healing (Tables, 2 and 3).

It was concluded that Mastic produced highly significant difference over placebo in healing of duodenal ulcer (P value less than 0.01) and also in relieving symptoms of duodenal ulcer (P value less than 0.01). The data were analysed using Z statistic method (Hunstberger, 1968).

Accordingly, it seems that Mastic is effective in relieving symptoms of duodenal ulcer and in promoting its healing.

The drug was free from side effects because no patients developed untoward clinical effects or deviation of the normal function of vital organs as estimated by laboratory tests done in patients with benign gastric ulcer treated by Mastic in an open trial during the same period (Al-Habbal and Huwez, 1983).

This new drug is of plant origin and is a resinous exudate from the plant *Pistacia lentiscus* (Family; Anacardiaceae) which is cultivated in the Mediterrenean countries particularly in the Greecian Archipelago specially on the island of Scio (Bail, 1935). Mastic is extracted from the tree *Pistacia lentiscus* by making long incisions on the trunk and larger branches from which the resinous juice exudes and collect on the outside of the tree where it hardens (Claus *et al*, 1970). Mastic pieces are of variable sizes and shapes and hard in consistency; brittle, pale yellow clear and glassy when fresh but dull and dusty if stored, possessing an aromatic odour with an agreeable taste (British Pharmaceutical Codex, 1949). The acid value is not more than 70 and its melting point ranges between 105 and 120 degree Centigrade, and it is insoluble in water while partially soluble in alochol and oil of turpentine but it is very

soluble in chloroform (2/1) and in ether (2/1) (British Pharmaceutical Codex, 1949).

The chemical composition of Mastic is not similar to the currently used anti-ulcer drugs because Mastic is composed of more than 90% of resins, 2% volatile oil and a bitter principle (Claus *et al*, 1970). The volatile oil is composed of d-*alpha*-Dipnene and gives the balsamic odour to the drug (British Pharmaceutical Codex, 1949). The resins of Mastic are composed of *alpha* and *beta* masticonic acid (38%), *alpha* and *beta* masticinic acid (4%), *beta* masticoresene which is insoluble in alcohol (30%) and masticolic acid (British Pharmaceutical Codex, 1949).

The ideal drug for treatment of duodenal ulcer is that drug which relieves symptoms, heals ulcer and keeps them healed. Because of break through recurrences and post treatment relapses with cimetidine, another drug with lower incidence of break through recurrences and post-treatment relapses is actively sought for to replace cimetidine (Wormsley, 1980). However, in this preliminary report it is not possible to evaluate Mastic effect in prevention of post-treatment relapses because the patients were not followed after they ended the treatment and the trial for long periods of time by post-trial duodenoscopic examinations. Yet, even if relapses occur, Mastic is worthy because the duration of treatment was short (two weeks) and it was given in single daily dose of 1 gram.

From the results of this preliminary report, other studies are needed regarding the mode of action, the active ingredients, the pharmacodynamics and pharmakinetics of the drug and other clinical randomized multi-centre double blind control trials versus placebo and/or other known anti-ulcer drugs.

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TABLE 1

NUMBER OF PATIENTS IN BOTH GROUPS WITH THEIR SEX AND AGE DISTRIBUTION

GROUP	TOTAL No. OF PATIENTS	MALE	FEMALE	RANGE OF AGE (YEARS)
MASTIC	20	18	2	27-62
PLACEBO	18	15	3	22-55

TABLE 2

SYMPTOM RELIEF

GROUP	TOTAL No. OF PATIENTS	No. OF PATIENTS WITH SYMPTOM RELIEF	PERCEN-TAGE
MASTIC	20	16	80%
PLACEBO	18	9	50%

(P value less than 0.01) using Z statistic method

TABLE 3

ENDOSCOPIC HEALING

GROUP	TOTAL No. OF PATIENTS	No. OF PATIENTS WITH ENDOSCOPIC HEALING	PERCEN-TAGE
MASTIC	20	14	70%
PLACEBO	18	4	22 %

(P value less than 0.01) using Z statistic method

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MASTIC IN TREATMENT OF BENIGN GASTRIC ULCERS

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MASTIC IN TREATMENT OF BENIGN GASTRIC ULCERS*

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IRAQ

INTRODUCTION

Mastic is a resinous exudate from the plant *Pistacia lentiscus* which belongs to the family Anacardiaceae and is cultivated in the Mediterranean countries particularly in the Greecian Archipelago and in the Eagean sea[1].

The chemical composition of Mastic is not similar to any other anti-ulcer drugs because it is composed of resins which constitute more than 90% volatile oil and 2% a bitter principle[2].

Oriental women had used Mastic since long-times as masticatory[3] and as breath sweetener[2].

Mastic is also used it in many parts of Mediterranean and some of European countries as a part of food and flavouring agent in cakes, icecreams, sweets and drinks[4]. In their report on the Review of Flavourings in food (1976), "Food additives and contaminants committee" of Ministry of Agriculture (UK), Fisheries and Food - stated that Mastic is acceptable and safe for use in the foodstuffs as a flavouring agent.

Medical uses of Mastic prior to our work on peptic ulcer included:

(1) Temporary filling carious teeth, preserving the teeth and sweetening the breath[5,6].

(2) Compound Mastic Paint - as a protective covering for wounds and to hold gauze in position[5].

(3) It is kept in mouth for sore mouth and cure of aphthae[6].

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Since a long time ago Mastic had been used by the public and local Traditional Healers in many parts of the Mediterranean area for relief of upper abdominal pain and heart burn which probably originated from the Arabic Medicine in the tenth century and afterwards because Mastic had been mentioned by the famous Arab physicians (Ibn Al-Jazzar and Ibn Al-Baytar) for the treatment of gastric ulcers[7,8] and for intestinal ulcers[8].

Recently, by using a double blind controlled clinical trial in Arbil Teaching Hospital (North of Iraq), Mastic proved to have statistically significant effect in relieving symptoms and healing of duodenal ulcers over placebo[9]. This prompted us to use it in the treatment of benign gastric ulcer as well. This study was done at Arbil Teaching Hospital.

PATIENTS AND METHODS

Mastic extract was used in the treatment of six patients with benign gastric ulcers which were proved both endoscopically and histologially in an open clinical trial after taking their informed consents. One patient had double gastric ulcers. Two patients did not respond to several months therapy with cimetidine. All the patients were above 20 years of age (five male and one female). The female patient was 70 years old diabetic with ischemic heart disease and atrial fibrillation and she did not respond to several courses of treatment with cimetidine. All other patients had neither clinical nor laboratory evidence of other diseases. They had not received recent treatment within the previous two months with H_2 - blockers, bismuth, carbenoxolone or sucralfate. Mastic extract (in the form of powder) was given in a dose of one gram twice daily (one dose before breakfast and the other at bed time) for four weeks. Routine laboratory investigations including general urine examination and complete hematological and biochemical profiles were done at 0,2 and 4 weeks during the course of the treatment and

monthly thereafter for two months after the course of the treatment. Endoscopic follow up was done every two weeks by the same physician (FUH), and the endoscopic findings were recorded on Video tape film. Endoscopic healing was defined as complete epithelisation of the ulcer without appearance of other new ulcers[10]. The patients were told to give up smoking, avoid fried food and anti-inflammatory drugs. They were allowed to take antacid tablets (Gastrigel tablets) on demand for relief of upper abdominal pain. The clinical and laboratory evaluations and follow up were done by the other author (MJH).

RESULTS

Complete symptomatic relief was found in all the patients in a mean duration of seven days after commencement of the therapy. Endoscopic healing was found in five patients (including the patient with double gastric ulcers and the elderly female patient) at the end of the four weeks of the treatment with mastic extract.

Neither clinical side effects nor abnormalities in the laboratory indices were observed during the course of the treatment and two months afterwards.

DISCUSSION

The dose of Mastic extract used in this study did not exceed the quantities used by the public as masticatory gum, breath sweeteners, or food flavouring. There are no reports of side effects to Mastic, neither from non-medical users, nor in the pharmacognosy books and Encyclopedias of drugs [2,3,5,11].

In an earlier doubled blind controlled trial, it was found that Mastic is useful in the treatment of duodenal ulcer and had no side effects[9].

In this report, the number of patients is small (six cases), because gastric ulcer is not common in Arbil area; 14 cases only (3%) were documented by the authors amongst 463 patients who

underwent upper G.I.T. endoscopy during one year period[12]. However, the observation that all six patients showed symptomatic relief and five of them had complete endoscopic healing at the end of the treatment, suggests that Mastic may be useful in treating gastric ulcers. Double blind controlled trials including larger number of patients are planned to see if Mastic can increase the rate of peptic ulcer healing and prevent relapse.

The mechanism of action of Mastic introducing symptomatic relief and ulcer healing is not known. However, Mastic is not soluble in water and therefore we raise the possibility that Mastic may form complexes with proteins and produce cytoprotective layer which protects gastric mucosa from injurious agents (such as bile salts and acid pepsin).

More studies are going on to investigate the pharmacodynamics and pharmacokinetics of Mastic to establish its role in the treatment of peptic ulcers.

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PROTECTION OF GASTRIC MUCOSA BY ALOE VERA

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PROTECTION OF GASTRIC MUCOSA BY ALOE VERA*

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INTRODUCTION

Peptic ulcers are very common. It is estimated to be present in 5.8% of men and in 1.9% of women in the population. The lesions are ulcerations of the mucosa and underlying structures, usually of the lesser curve of the stomach or first part of the duodenum. The ulcers usually start as an acute form when they are small and multiple and surrounded by petechial hemorrhages. They tend to heal readily unless there is gastric stasis and hyperacidity when they become chronic and erode the wall of the gastric mucosa (Warner, 1964).

The gastric mucosa is remarkably resistant to injury. Two factors normally protect the stomach from autodigestion, namely the gastric mucosa and the epithelial barrier. The application of an irritant to the gastric mucosa is followed by outpouring of large quantities of mucus that owes its protective capacity to its physical characteristics and its ability to absorb pepsin. The epithelial lining has remarkable properties of repair and is able to reproduce itself within 36 to 48 hours (Silen, 1974).

A peptic ulcer is the result of a continued action of the gastric juice on an area of mucosa which is presumably of lowered resistance. There are three causes for this low resistance, namely - neurogenic, chemical

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or infective causes. The neurogenic causes result in abnormal vagal impulses from the hypothalamic region. This leads to vascular spasm and ischemia which may proceed to necrosis.

In addition, the vagal stimulation will lead to hypersecretion of the gastric juice. Chemical agents like cortisone and acetyl salicylic acid can lower the mucosal resistance by producing qualitative changes in the mucus and decreasing its total output (Boyd, 1961).

On the other hand, *Aloe vera* is known in folk medicine to possess a satisfactory healing capacity. The legend of this herb started in ancient Egypt thousands of years ago. It was specified in Ebers papyrus which was documented in the first dynasty 2270 years B.C. (Kamal, 1964).

Recently, el-Zawahry and Hegazy (1970) demonstrated the therapeutic value of *Aloe vera* in healing protracted skin ulcerations. This attracted our attention to explore any possible therapeutic or prophylactic effect of this plant against gastic ulcerations.

MATERIAL AND METHODS

Preparation of the *Aloe vera* pulp extract

Thirty leaves of the plant were cut with stainless steel knife and washed thoroughly with water. Then they were dried and kept for 24 hours in a vertical position to exclude the drained juice containing aloin. The edges of the leaves were cut, then they were split to curette the pulp which contain the mucopolysaccharide material. The pulp was mixed in a blender for one hour, sieved by fine gauze and kept in a refrigerator.

Implementation

Four animal groups of 12 male albino rats each (160-180 gm) were chosen to carry out prophylaxis and treatment experiments.

Induction of gastric lesions

This was performed for all the animals by applying a neurogenic and a chemical method simultaneously. Therefore, the animals were forcibly immobilised for 24 hours according to our method published two years ago (Galal *et al*, 1975). At the same time the animals received 100 mg/kg body weight of acetylsalicylic acid *per os*.

Prophylaxis groups

Each rat in the first group received 2ml of the extract twice daily *per os* for six days before induction of gastric lesions. The second group received only equimetric saline solution in the same manner. Then both groups were exposed to forcible immobilisation and chemical treatment for induction of gastric lesions.

Treatment groups

The third animal group received 2ml of the extract *per os* twice daily for six successive days after the immobilisation. The fourth group received saline in the same manner for the same period.

Recording of the gastric lesions was carried out for all the animals after incising the gastrum along the lesser curvature. The stomach was washed by saline and examined by the naked eye and the lens. Recording of the pH of the gastric juice was measured by pH meter (Beckman).

RESULTS

The results are shown in tables I and II.

TABLE I

THE PROPHYLACTIC EFFECT OF ALOE VERA (A.V.) ON INDUCED GASTRIC LESIONS BY 24 HOURS FIXATION AND ASPIRIN 100MG/KG IN RATS.

	Animal Groups	No. of Rats	No. of Lesions	Mean No. of Lesions/animal	Mean pH
I	Rats receiving A.V. for 7 days before induction of gastric lesions.	12	24	2	4.35
II	Control rats with induced gastric lesions.	12	160	133	4.30

Prophylaxis % = $\frac{136}{100}$ x 100 = 85%

TABLE II

THE CURATIVE EFFECT OF ALOE VERA (A.V.) ON INDUCED GASTRIC LESIONS BY 24 HOURS FIXATION AND ASPIRIN 100 MG/KG IN RATS.

	Animal Groups	No. of Rats	No. of Lesions	Mean No. of Lesions/animals	Mean pH
III	Rats receiving A.V. for 7 days after induction of gastric lesions.	12	42	3.5	5.23
IV	Rats receiving no treatment after induction of gastric lesions.	12	84	7	5.18

Curative % = $\frac{42}{84}$ x 100 = 50%

DISCUSSION

The results show that *Aloe vera* extract possesses a reliable prophylactic potential against this particular model of gastric lesions induced by forcible immobilisation and acetyl salicylic acid administration. If complete prophylaxis against gastric lesions is

referred to as 100%, the extract of *Aloe vera* was able to produce 85% prophylaxis. On the other hand, if complete curative treatment is presented by complete absence of gastric lesions, the *Aloe vera* extract produced a curative treatment equal to 50%. Gastric lesions induced by this method are probably due to two main etiological factors namely neurogenic and chemical irritation.

The brunt of nervous stress according to many workers may attack the gastric blood vessels. This condition is manifested by increased capillary fragility and permeability leading to petechiae, hematomata and even thrombosis. Consequently the gastric mucosa will be exposed to a degree of ischemia which lowers its resistance (Bourne, 1953; Coligado and Flesher, 1967).

When the chemical factor in the form of acetyl salicylic acid was added to the nervous stress, the gastric lesions were intense. They took the form of large hemorrhagic spots, ulcers and hemorrhagic streaks, as seen in the results.

It is most likely that the prophylactic and curative effect of *Aloe vera* is due to its protection of the gastric mucosa and supporting its resistance against the sequelae of chemical and nervous stress, without interfering with the gastric pH.

CONCLUSION

Aloe vera extract was successful as a prophylactic measure against gastric lesions induced by chemical and nervous stress in rats.

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ANTI-MICROBIAL AGENTS IN ISLAMIC MEDICINE

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ANTI-MICROBIAL AGENTS IN ISLAMIC MEDICINE*

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INTRODUCTION

Medicinal plants have been used during the ages for the cure and treatment of diseases but it was during the Islamic Era that the concept about anti-microbial agents was born. The Arabian physicians not only described the infections of the body but mentioned several medicinal plants and vegetable substances against rabies and hydrophobia in their pharmacopoeias[1] or medical formularies[2]. The use of medicinal plants against rabies and other infections during Islamic Era shows that the same possess some anti-microbial/anti-viral properties.

The Islamic medicine which is mostly based on herbal medicine is known to be catering to more than 80% of the population in the regions comprising the Muslim world. Since the infectious diseases are mostly prevalent in these regions, there is a need to develop some anti-bacterial agents of plant origin - comparable to antibiotics considering the local conditions and resources of these countries. Antibiotics are imported in these countries at the expense of foreign exchange. Research has already confirmed that higher plants possess considerable anti-bacterial activity when compared to modern antibiotics like chloramphenical and streptomycin[3]. Thus the study started in 1973 on 1500 varieties of higher plants available in U.S.S.R., Turkman, S.S.R. and N. Caucasus showed that plants were rich source of antibiotics[4].

* Bulletin of Islamic Medicine, 2: 496 - 499, 1982.

Many plants claiming to possess anti-dysentric, anti-septic, germicidal, fungicidal properties[5] and those considered to be effective against such diseases of microbial etiology like small-pox, tuberculosis, typhoid, diptheria[6] etc. are reported in the literature on traditional medicine. Similarly plants with anti-bacterial and anti-viral activity[7] have also been described in the literature. Considering that Islamic countries where traditional medicine is being practised are rich in medicinal plant resources, research efforts should be intensified to find out useful anti-microbial agents also because such agents are considered to be less toxic as compared to modern antibiotics because of their intimate connection with the nature.

Being prompted to discover some anti-microbial agents of plant origin, we in the National Institute of Health, Islamabad, started a screening program for monitoring anti-bacterial activity in medicinal plants in collaboration with P.C.S.I.R. Laboratories, Peshawar. The ultimate object of the study is to develop some dosage forms of herbal origin with antimicrobial activity comparable to modern antibiotics.

MATERIALS AND METHODS

Preparation of Extracts

Plants were cleaned, dried at a room temperature, powdered and the powders were extracted in soxhlet with ethanol. Solvent was removed and the extracts after fractionation with chloroform were used for the test.

Test Organisms

In our investigation the following micro-organisms were used:

Vibrio cholera, E. coli, Bacillus subtilis, Staphylococcus aureus, Shigella dysenteriae and *Salmonella typhi.*

Preparation of samples for testing

Tween 80 was used as solvent vehicle for the plant extract. All extracts were dissolved in the aforementioned solvent to give a concentration of 10mg/ml.

Anti-microbial testing

The conventional cup-plate diffusion method was used to test the anti-microbial activity of plant extracts. Molten nutrient agar was poured into petridishes as a basal layer and when it got solidified, seeded agar was poured over it. The agar was left to set after which 8mm core of agar was removed carefully with the help of a sterilized cork-borer from six peripheral positions and one central. The wells thus formed were aseptically filled up with the samples. The central hole was filled with Tween 80 as a blank. After holding the petridishes for two hours in the same position they were incubated for 18-24 hours at 37°C and zones of inhibition produced by plant extracts or otherwise were recorded after the incubation period. Those extracts which showed no zones of inhibition were denoted with negative (-) sign while those producing zones of inhibition were denoted with positive (+) sign which were measured in cm by vernier calliper. Extracts were denoted with + or + + signs depending on the size of zone of inhibition.

RESULTS AND DISCUSSION

Primary screening results of anti-microbial activity are given in Table 1. As can be seen, out of 22 plants screened, 12 have shown interesting activity. It is evident from the above study and work carried out elsewhere that higher plants could offer a good potential for the development of anti-biotic drugs. In fact plants like *Psoralea corylifolia*[8], *Myrtus communis*[9], *Nigella sativa*[10], *Glycyrrhiza glabra*[11], *Cannabis sativa*[12], *Jetropha podagrica*[13], and many others used in the Islamics medicine for different purposes are lately reported to possess anti-microbial properties but they are not used

in therapeutics for the above purpose. It was in view of the above consideration that the proposed study was undertaken.

In the first paper published in Fitoterapia in 1980 we described the results of anti-microbial activity of 71 extracts obtained from 26 species of medicinal plants[14]. Subsequently, another paper was published in the same journal reporting the results of anti-microbial activity of 90 extracts obtained from 33 species of wild growing plants[15].

With the above study, anti-microbial screening of 81 plant/ species have been completed. These studies definitely show that some of the medicinal plants possess good anti-microbial activity when compared against modern antibiotics. Being encouraged with these observations, it is worthwhile to prepare some dosage form out of the plants screened. For this purpose, *Myrtus communis*, the essential oil of which had exhibited good anti-bacterial activity specially against *E. coli* and *Shigella dysenteriae* in a previous study, was selected. This plant is commonly used as such in traditional medicine for different purposes.

An anti-diarrhoeal oral emulsion and a cream for topical use were prepared from the essential oil. While the stability and toxicological study of the oral emulsion is still in progress, the topical cream in a 5% V/W concentration demonstrated good anti-bacterial activity comparable to Furacin cream of S.K. & F. However, the work is still in progress which will be presented in some later publication.

CONCLUSION

Medical plants used in the Islamic medicine offer a great reservoir for the discovery of new anti-microbial drugs comparable to anti-biotics used in modern medicine. Since almost all the anti-microbial agents are being imported into the countries comprising the Islamic world and considering the local availability of medicinal plants in these countries, it is imperative that a serious scientific effort should be made to find out new anti-microbial drugs of herbal origin relevant to the disease pattern in these countries.

TABLE 1

ANTI-MICROBIAL ACTIVITY OF SOME WILD GROWING PLANTS

S.No.	NOMENCLATURE	*Activity against*					
		B. subtilis	***E. Coli***	***Vibrio cholera***	***Sh. dysenteriae***	***Staph. aureus***	***Sal. typhi***
1.	*Arenaria leptoclados* Guss. st, lf.	-ve	-ve	-ve	-ve	-ve	-ve
2.	*Argyrolobium roseum*, CAMB, st.	-ve	-ve	+ve	-ve	-ve	-ve
3.	*Dianthus crinitus* rim. st. lf.	-ve	+ve	+ve	-ve	+ve	-ve
4.	*Polygala hohenacke-riana*, Fisch & May, st. lf	-ve	+ve	+ve	-ve	-ve	-ve
5.	*Saussurea heteromalla*. D.Don, st. lf.	++ve	++ve	++ve	++ve	++ve	++ve
6.	*Impatiens balfourii* Hk. st. lf	-ve	+ve	+ve	+ve	+ve	-ve
7.	*Dipsacus mitis*, st. lf.	-ve	+ve	-ve	+ve	+ve	-ve
8.	*Urtica dioca* st. lf	-ve	+ve	-ve	-ve	-ve	-ve
9.	*Tagetes patula* L. st, lf, Fr.	-ve	+ve	-ve	-ve	+ve	-ve
10.	*Kickxia incana* Wall st. lf	-ve	+ve	-ve	-ve	+ve	-ve
11.	*Spiraea vaccini-polia* D. Don, st. lf.	-ve	+ve	+ve	-ve	+ve	-ve
12.	*Salvia moorcroftiana*, st, lf, Rt.	-ve	+ve	+ve	-ve	+ve	+ve
13.	*Hedera nepalensis* K. Kock, lf, Rt.	+ve	+ve	+ve	+ve	-ve	+ve
14.	*Anemone obtusiloba* D. Don. st. lf.	-ve	-ve	-ve	-ve	-ve	-ve
15.	*Oxalis pescaprae*. st. lf.	-ve	-ve	-ve	-ve	-ve	-ve
16.	*Lithospermum qrvense* L. St, lf.	-ve	-ve	-ve	-ve	-ve	-ve
17.	*Reseda aucheri* Boiss. st, lf	-ve	-ve	-ve	-ve	-ve	-ve
18.	*Silene viscosa* (L) Pors st, lf.	-ve	-ve	-ve	-ve	-ve	-ve
19.	*Silena conoidea* L. st, lf.	-ve	-ve	-ve	-ve	-ve	-ve
20.	*Convolvulus glomeratus* Clarke st, lf.	-ve	-ve	-ve	-ve	-ve	-ve
21.	*Reinwardtia indica* Du Most. st.	-ve	-ve	-ve	-ve	-ve	-ve
22.	*Potamogeton indicus* st.	-ve	-ve	-ve	-ve	-ve	-ve

NOTE:-

i) Tween - 80 was used as solvent.

ii) Abbreviations used: st. (stem), lf (leaf), rt (root).

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RESEARCHES ON THE ANTIMICROBIAL ACTIVITY OF THE VARIETIES OF GLYCYRRHIZA GLABRA GROWING IN TURKEY

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TURKEY

RESEARCHES ON THE ANTIMICROBIAL ACTIVITY OF THE VARIETIES OF GLYCYRRHIZA GLABRA GROWING IN TURKEY*

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Glycyrrhiza glabra L. is an economically valuable plant which has been much studied, even in quite recent years. The extracts of licorice roots have been used for various purposes in curing a great deal of illness in both folk medicine and medical treatment. Even nowadays they are being used[1].

In our previous researches, according to our morphological and anatomical studies on *G. glabra* grown naturally and widespread especially over the East, South, and South-east of Anatolia, we have distinguished 4 varieties such as : *G. glabra var. glandulifera form (a), - form (b), - var. glabra and var. violaceae*[1]. And also we have determined and compared the chemical constituents of the roots of these varieties which we have distinguished[2].

This time, in this study, the antimicrobial activity of the Turkish varieties were detected and also compared with the activity of *G. glabra var. typica* which is known in commerce as Spanish licorice.

The samples on which we have studied, were collected from the fields along the roads and sandy places of stream and river beds by digging up the ground at a depth of 0.5-1 m. After removing the overground parts of the plant, the underground parts were air-dried and powdered.

1- A serial extraction was applied to these powdered samples (Scheme 1).

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* Bulletin of Islamic Medicine, 3: 365 - 371, 1984.

2- Then the solutions of the samples at a concentration of 100 mg/ml in dimethyl-sulfoxide (DMSO) were prepared from each fraction. And the Minimum Inhibitory Concentration (MIC) of these solutions were determined at 100 and 1000 µg/ml level by agar-dilution streak method.

The microorganisms that have been used during the activity test were:

- *Staphylococcus aureus*
- *Escherichia coli*
- *Salmonella gallinarum*
- *Klebsiella penumoniae*
- *Mycobacterium smegmatis*
- *Candida albicans*

The varieties which are growing in Turkey and on which we have studied, showed antimicrobial activity *in-vitro* against only 2 of these microorganisms. They were *Staphylococcus aureus* and *Mycobacterium smegmatis*. However Spanish licorice was also active against *Candida albicans* besides these 2 microorganisms (Table 1).

3- The majority of the biological activity was found in MeOH extract (supposed to be the neutral terpene-steroid fraction). So the residue of this bioactive fraction was dissolved in $CHCl_3$ and the $CHCl_3$ solution was applied to a silicic acid column packed in $CHCl_3$ (sample: adsorbent ratio was 40:1 and diameter: height ratio was 14:1). The column was first eluted with $CHCl_3$ and then with different percentage of MeOH and $CHCl_3$. And the collected fractions were applied to TLC. The adsorbent was silicagel G/ UV_{254} (Polygram precoated 0.25 mm plates) and the eluting solvent was MeOH:$CHCl_3$ (5:95). The fractions that showed the same spots were combined to make new fractions.

Both the new fractions combined after TLC and the total mixture of these fractions and also the authentic samples were separately applied to HPLC under the same conditions. And the retention times of these solution were determined individually.

As Column: ODS (Excalibar) reverse phase was used.

Mobile phase was MeOH: H_2O (A%:50), gradient system was applied at the beginning.

A% flow rate: 2 ml/min.

Total flow rate: 2ml/min.

In addition to the detection of the retention times, the solution of the authentic samples and the total fraction was injected to the column together. In this manner, the bioactive compounds of isoflavan structure such as: glabrene, 3'-methoxyglabridin and also in smaller-quantities hispaglabridin A and B, 4'-o-methyl glabridin, glabrol (flavanone), phaseollin-isoflavan have been confirmed (Formula 1). Besides, it was seen that one of the obtained peaks was identical to formononetin which is antimicrobially inactive.

Finally, when HPLC chromatogram were examined, it was understood that Turkish samples do not contain "Glabridin" which is active against *Candida albicans* and which is found in commercial Spanish licorice *(-var.typica)* (Chrom. 1, 2, 3, 4, 5). This also proved the antimicrobial activity test[3,4].

It is obviously seen that the formononetin peak is higher than the peaks of the other compounds identified both in Turkish and Spanish licorice samples (Chrom. 3, 4, 5).

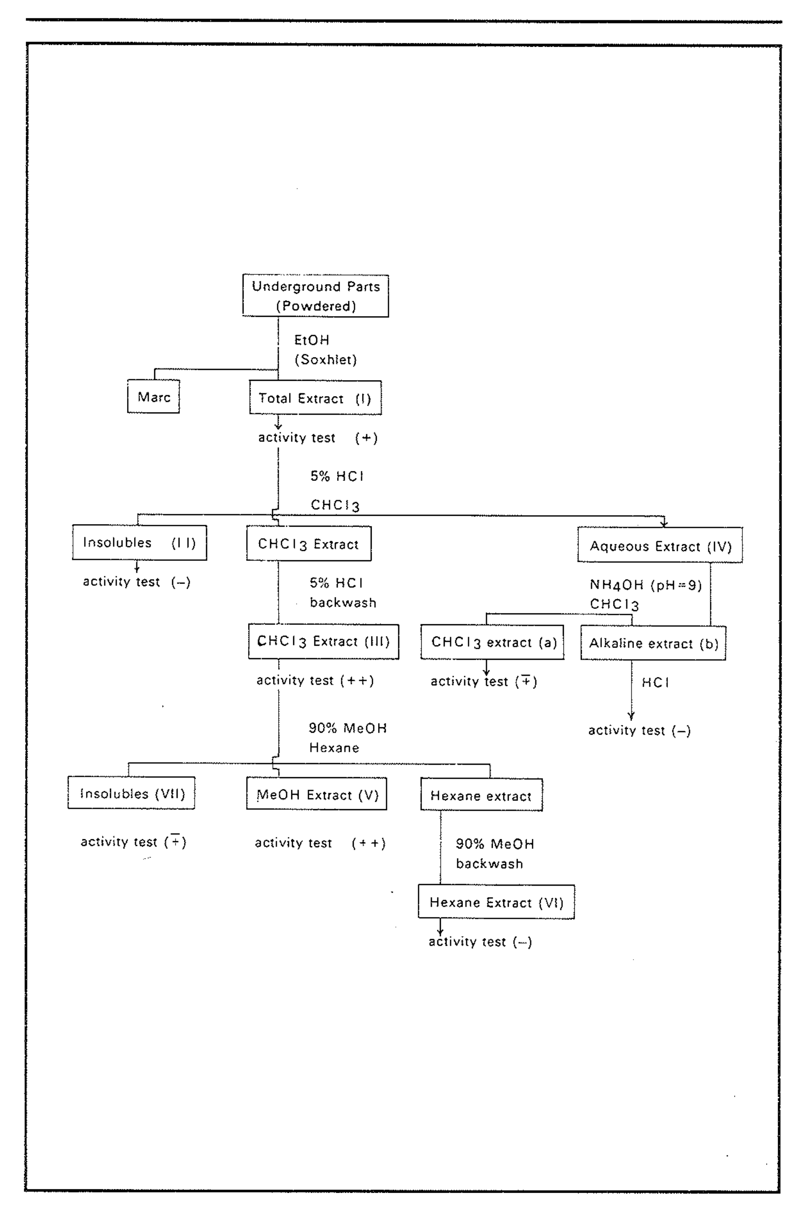
And again, when we look at the chromatograms of Turkish samples, it is observed that the higher peaks belong to 3'-methoxyglabridin and glabrene (Chrom. 1, 2, 3). On the contrary to this, hispaglabridin A and B peaks are higher on the chromatogram of Spanish sample (Chrom. 5). It was also understood that 3' methoxyglabridin is the major bioactive compound of Turkish samples as its peak was the highest of the others.

By the HPLC seperation of the MeOH extracts of both Spanish and Turkish samples, we obtained 2 small peaks which were identified as O-acetyl salicylic acid and salicylic acid by comparison with the authentic samples. Before our research, these 2 compounds have been first determined in commercial Spanish licorice roots' extract[4]. Curiously, aspirin seems not to have been encountered directly in nature before. As the peaks were too small, the quantity present in the plant might be relatively small but its presence in licorice extracts might lead to some therapeutic effect.

On the chromatogram of the MeOH extract of *G. glabra var. glandulifera form (b)* (One of Turkish samples), there are two unknown peaks that we have not identified yet (Chrom 2).

As a result of our studies we have detected that all Turkish licorice varieties *[G. glabra var. glanduliefera form (a), -var. glandulifera form (b), -var. glabra* (samples collected from Mus and Tatvan)] show antimicrobial activity which equals that of Spanish licorice, against *Staphylococcus aureus* and *Mycobacterium smegmatis*.

Moreover, our studies on Turkish *G. glabra var. glandulifera* have indicated that this variety also has antimicrobial activity, although it was reported in one of the research papers that *G. glabra var. glandulifera* known as Russian licorice is biologically inactive against the microorganisms[3,4].

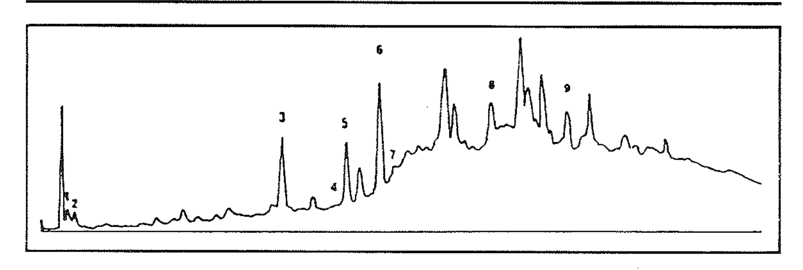


Scheme 1. Extraction of the underground parts of the varieties of *Glycyrrhiza glabra*.

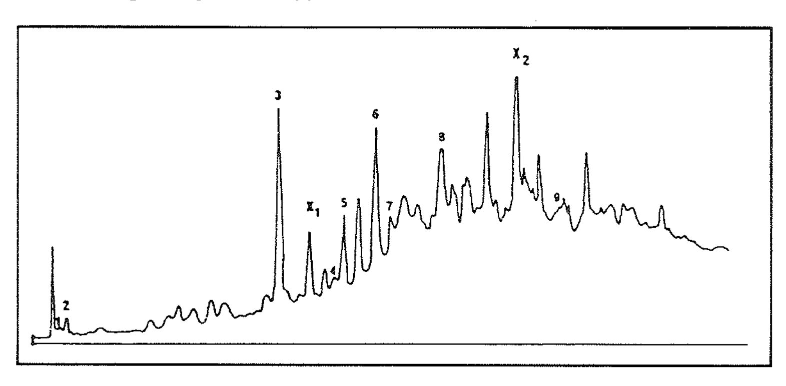
TABLE 1: *IN-VITRO* ANTIMICROBIAL ACTIVITY OF THE DIFFERENT FRACTIONS OF THE VARIETIES OF *G. GLABRA*.

Varieties	EtOH extr. (I)	Ins. $CHCl_3$ extr. (II)	$CHCl_3$ extr. (III)	Aq. ext (IV)		MeOH extr. (V)	Hex. extr. (VI)	Ins. (MeOH) extr. (VII)	Micro-organ-isms
				$CHCl_3$ extr. (a)	Alkal. extr. (b)				
G. glabra var. glandulifera form (a)	1000	-	1000	-	-	1000	-	-	*S. aureus*
	1000	-	< 100	1000	-	< 100	-	1000	*M. smegmatis*
	-	-	-	-	-	-	-	-	*C. albicans*
G. glabra var. glandulifera form (b)	1000	-	< 100	-	-	< 100	1000	< 100	*S. aureus*
	1000	-	< 100	1000	-	< 100	1000	1000	*M. smegmatis*
	-	-	-	-	-	-	-	-	*C. albicans*
G. glabra var. glabra (Mus)	1000	-	< 100	-	-	< 100	-	-	*S. aureus*
	1000	-	< 100	1000	-	< 100	-	1000	*M. smegmatis*
	-	-	-	-	-	-	-	-	*C. albicans*
G. glabra var. glabra (Tatvan)	1000	-	1000	-	-	< 100	-	-	*S. aureus*
	1000	-	< 100	1000	-	< 100	-	± 1000	*M. smegmatis*
	-	-	-	-	-	-	-	-	*C. albicans*
G. glabra var. typica (Ticari Ispanya meyani)	-	-	< 100	-	-	< 100	-	-	*S. aureus*
		-	< 100	-	-	< 100	-	± 1000	*M. smegmatis*
		-	1000	-	-	1000	-	-	*C. albicans*

Minimum Inhibitory Concentration has been tested at 100 and 1000 $\mu g/ml$ levels.

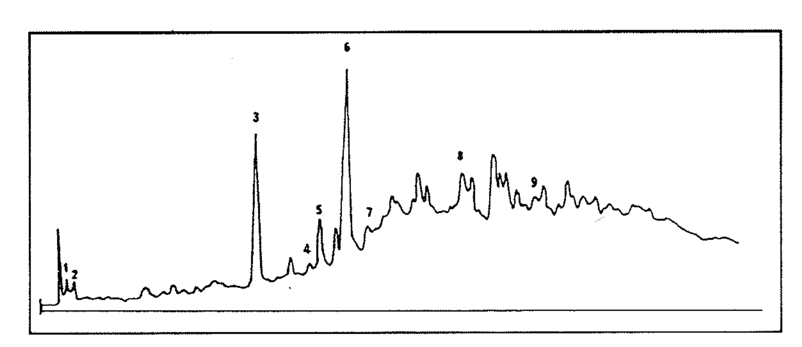


Chrom. 1: HPLC Chromatogram of the MeOH extract of *G. glabra var. glandulifera* form (a)

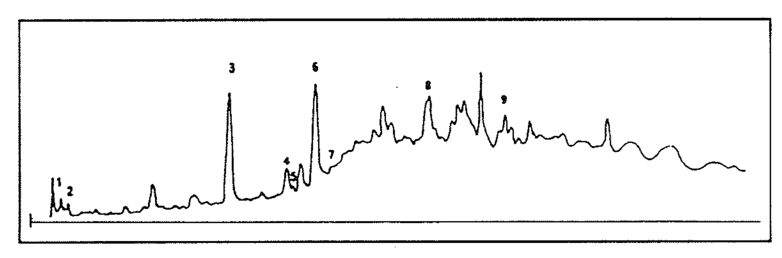


Chrom. 2: HPLC chromatogram of the MeOH extract of *G. glabra var. glandulifera* form (b).

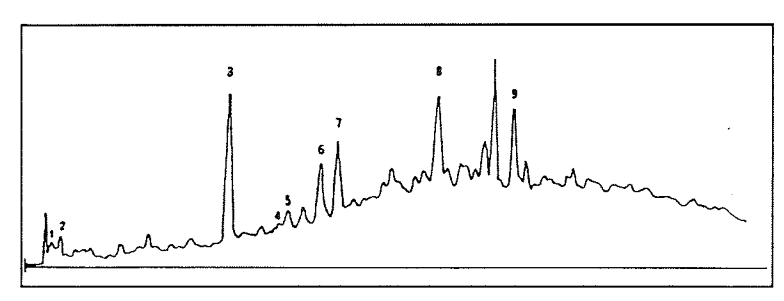
(1) O-acetyl salicylic acid
(2) Salicylic acid
(3) Formononetin
(4) Phaseollinisoflavan
(5) Glabrene
(6) 3'-Methoxy glabridin
(7) Glabridin
(8) Hispaglabridin A Glabrol 4', O-methylglabridin
(9) Hispaglabridin B



Chrom. 3: HPLC chromatogram of the MeOH extract of *G. glabra var. glabra* (Mus.).



Chrom. 4: HPLC chromatogram of the MeOH extract of *G. glabra var. glabra* (Tatvan).



Chrom. 5: HPLC chromatogram of the MeOH extract of *G. glabra var. typica* (Commercial Spanish licorice).

Hispaglabridin A

Hispaglabridin B

4',O-methylglabridin

Glabridin

3',methoxyglabridin

Glabrol

Glabrene

Formononetin

Formula 1

Phaseollinisoflavan

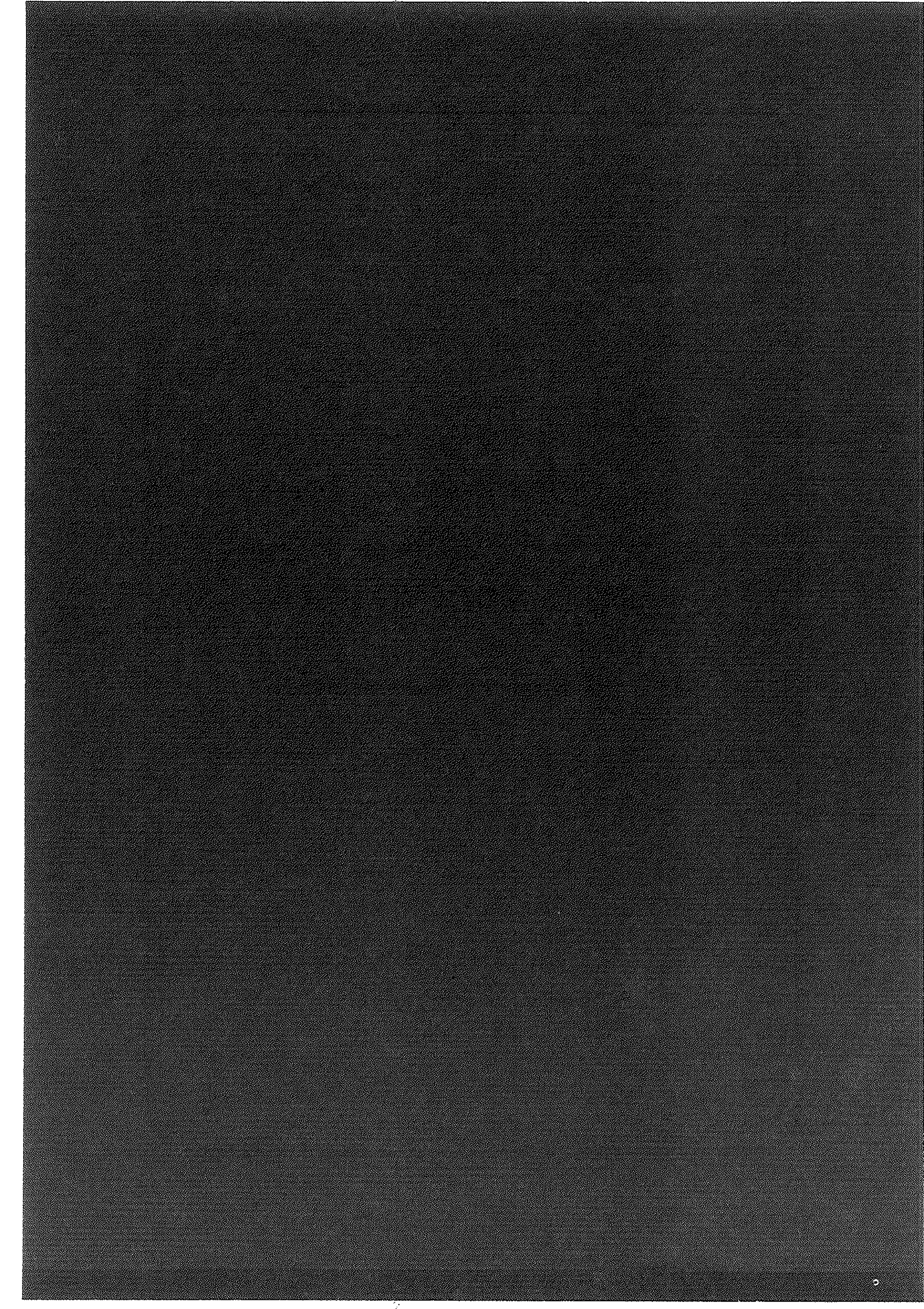
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CASSIA IN ISLAMIC MEDICINE AND ITS MODERN USES

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INDIA



CASSIA IN ISLAMIC MEDICINE AND ITS MODERN USES*

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Abstract

The genus *Cassia* of the family *Leguminosae* is a famous medicinal plant, which has been in use in Islamic Medicine. The word *'senna'* often used with *Cassia* is the Latin form of Arabic *'sena'* or *'sana'*. The plant is used since long for the purgative properties of the leaves. Pulp is used against worms and cough. In case of sorethroat the seeds are used for gargle. A blood purifier drug SAFI (product of Hamdard Wakf. Labs., Delhi) contains concentrated aqueous extracts of *Cassia angustifolia (senna)* and *C. occidentalis (kasaunki)* as ingredients.

Based on its Islamic medicinal background we have tried to analyse its properties with modern biochemical methods and have found antiviral principles in several of the species of this plant. These aspects have been discussed in further detail here.

INTRODUCTION:

The genus *Cassia* of the family *Leguminosae (Caesalpinaceae)* is a well-known medicinal plant (Chopra *et al*, 1956, Satyavati *et al*, 1976, Levis and Elvin-Lewis, 1977), particularly in the Islamic system of medicine (Bhandari 1959, Misra and Sinha 1978). We have tried to evaluate whether the 8 species of the genus growing in this area (Sinha, 1976) have antiviral properties. The therapeutic value of *Cassias* has been recognised in several

* Bulletin of Islamic Medicine, 1: 390 - 394, 1981.

systems of medical practise. In Islamic system of treatment *C. angustifolia, C. fistula, C. occidentalis, C. sophera* and *C. tora* have been recognised as medicinals (Anonymous 1959). Flowers and pulp of *C. fistula* are useful as purgative and in the case of cough. Pulp is also used against worms. In case of sore throat, seeds and pulp are used as gargle. Ash of fruit with common salt and honey cures cough. *C. occidentalis* and *C. sophera* are useful in snake poisoning. Root of the plant with golmirch *(Piper nigrum)* is used successfully as antidote to cold. Leaves are also said to cure the inflammation of the heart. In case of toothache, a paste of fresh root in water is used. Herbaceous *C. tora* is useful as blood purifier whereas seeds are used to cure cough and asthma. In chronic skin diseases as ringworm, itching etc., a paste of seeds are used with lemon juice, both internally as well as externally. *C. angustifolia* is also mentioned as purgative.

A general blood-purifier drug, SAFI, a product of Hamdard (Wakf) Labs., Delhi contains concentrated aqueous extracts of *C. angustifolia* and *C. occidentalis* as ingredients.

All the aspects need many investigations over several years which is being tried. However, we discuss here the antiviral properties of the 8 *Cassia* species growing in our area.

MATERIALS AND METHODS

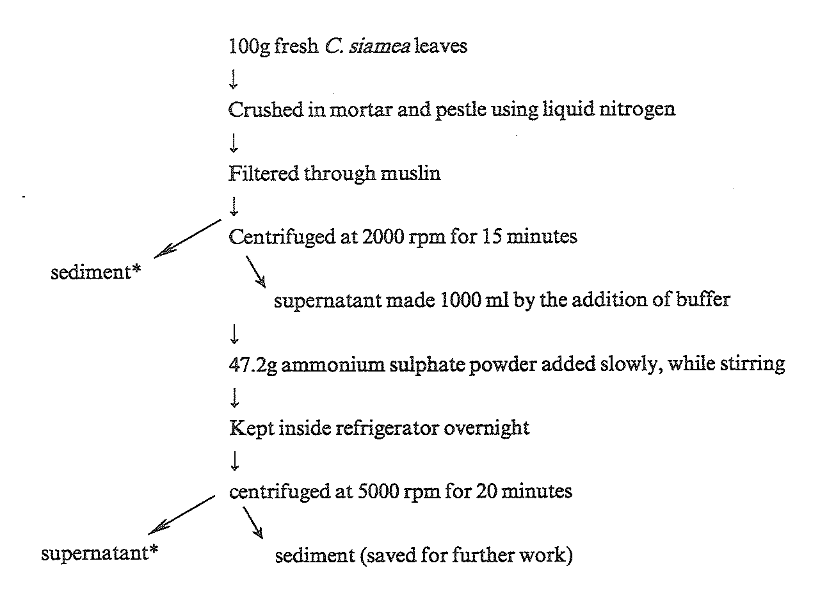
The following species of *Cassia,* collected from the local area were examined. *C. fistula* Linn, *C. glauca* Lamn., *C. marqlinata* Roxb, *C. nodosa* Buch. Ham., *C. occidentalis* Linn., *C. siamea* Lamk., *C. sophera* Linn., and *C. tora* Linn. *C. glauca* is shrub, *C. occidentalis, C. sophera* and *C. tora* are herbs while the other four species: *C. fistula, C. marqinata, C. nodosa* and *C. siamea* are trees.

Aqueous and benzene extracts of the leaves of different species were prepared separately and mixed with the suspension of TMV (Tobacco mosaic virus) in varying concentrations. The mixture was

applied to the surface of detached tobacco *(Nicotiana tabacum var. xanthi)* leaves incubated in humid chambers for 48-64 hours. The intensity of virus activity was evaluated by counting the number of local lesions developed on the test leaves.

Control was maintained by using TMV suspension mixed with buffer solution, instead of *Cassia* extracts. In each case the first half of the leaf was brushed with test material (TMV + *Cassia* extract) and the other half with control solutions (TMV + buffer).

The virus-inhibiting property of *Cassia siamea* was further investigated, as it proved to be most potent, as explained below (Misra, 1977; Misra and Sinha, 1978; Misra and Sinha, 1979).



* Discarded

The sediment was the protein component which was again used for bio-test against TMV.

RESULTS

The results have been indicated in the table provided. It is evident that *C. siamea* had the maximum inhibiting capacity for TMV. *C. fistula, C. glauca, C. occidentalis,* and *C. tora* had very little inhibiting capacity. *C. marginata* and *C. nodosa* had practically no effect. *C. sophera* was different from others and showed promotory effect on the viruses.

The benzene extract of different species of *Cassia* had almost the same activity, except that the performance of inhibition improved a little, in comparison to water extracts.

The protein precipitate extracted from *C. siamea* and used for bio-test against TMV, exhibited high degree of inhibition of local lesions, even up to 100% in some cases.

TABLE: Bio-test of water and benzene extracts of *Cassia* leaves, on *Xanthi tobacco*, against TMV (1:10)

SPECIES	WATER EXTRACT TEST/CONTROL		BENZENE EXTRACT TEST/CONTROL	
	No. of local lesions	% Inhibition	No. of Local lesions	% Inhibition
C. fistula	142/185	23.245	123/172	27.348
C. glauca	98/130	24.615	107/147	27.210
C. marginata	146/148	1.351	142/144	1.388
C. nodosa	157/158	0.623	149/153	2.614
C. occidentalis	124/170	27.058	117/168	30.357
C. siamea	36/166	78.318	36/178	79.775
*C. sophera**	128/153	19.431	171/140	22.222
C. tora	110/150	26.666	116/162	29.629

* *C. sophera* had promotory effect instead of inhibition. A drop of benzene was added in controls.

DISCUSSION

There have been many reports of anti-microbial activity of chemical compounds isolated from *Cassia* sps. (Mickell, 1959; Gaind *et al*, 1966; Lillykutty and Santhakumari, 1969). Fungicidal compounds have been known in C. fistula (Venkataraman and Radhakrishnan, 1972) and *C. tora* (Acharya and Chatterjee, 1974). These substances were identified as flavonoidal glycosides, and chrysophanic acid-9-anthrone respectively. Chaksine an alkaloid isolated from *C. absus* has been found responsible against bacteria (Gupta and Chopra, 1953). Insecticidal property of *Cassia* have also been reported by Rao (1957). Dhar *et al* (1968) have mentioned the use of *C. auriculata*, *C. fistula* and *C. tora* against Ranikhet and Vaccinia viruses. Other properties of *Cassia* as diuretic (Bhide and Seth, 1957), analgesic (Patel *et al*, 1965), cathartic (Iyengar *et al*, 1966), hyperglycaemic (Shrotri *et al*, 1963; Dhar *et al*, 1968) toxic (O'Hara and Pierce, 1974) etc. have also been noted. The laxative principles (Van Os, 1976) and the use of the genus *Cassia* in several types of skin diseases are well known since long (Chopra *et al*, 1956; Sharma and Das, 1976).

Naturally occurring virus-inhibiting principles in plants have been known since long, in several types of plants (Mathews, 1970). *Cassias* containing inhibitory principles against plant viruses has also now been established. It has further been proved that virus-inhibitor in *Cassia siamea* was proteinous in nature.

CONCLUSION

The ancient claim of medicinal properties of *Cassia* plants in Islamic medicine has been corroborated by modern scientific data, that it contains anti-viral proteins. *C. siamea* is more potent than other 7 species growing in this area. Seeds of *Cassia occidentalis* are widely used as a substitute for coffee in Egypt (Hassan *et al*, 1974). It is known as "Negro-Coffee", and has passed the organoleptic

tests. Promotion of *Cassia* as coffee may be advanced further due to the facts that they are of medicinal value also.

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USE, ABUSE AND PRESENT STATE OF SCIENTIFIC KNOWLEDGE OF KHAT

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I.A. Al-Meshal, M. Tariq,
N.S. Parmar and Abdulrahim Abujayyab.

SAUDI ARABIA

USE, ABUSE AND PRESENT STATE OF SCIENTIFIC KNOWLEDGE OF KHAT*

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SAUDI ARABIA

INTRODUCTION

The Prophet Mohammad (ﷺ) communicated the Islamic message to his followers, which are sacredly embodied in the Holy Book, "The Quran".

> *(THIS IS) A SCRIPTURE WHICH WE HAVE REVEALED ONTO THEE THAT THEREBY THOU MAYST BRING FORTH MANKIND FROM DARKNESS UNTO LIGHT, BY THE PERMISSION OF THEIR LORD, UNTO THE PATH OF THE MIGHTY, THE OWNER OF THE PRAISE.*

This verse from the Holy Quran clearly emphasises to bring about a significant change from pagan life to shining life of Islam. Following the teachings of 'Quran' that

> *THOU SHALL NOT DRINK WINE OR ANY-THING INTOXICANT*

Muslims were strictly forbidden to take any alcohol; however, consumption of Khat (a psychostimulant plant material) was controversial for a long time. According to some authors Khat enjoyed divine blessings and no private or public religious ceremony took place without ritual chewing of this leaf, and some Muslim communities regarded it as a gift from heaven. According

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to Laurent[1], while the Christians were allowed to consume alcohol, Muslims were devoted to Khat consumption.

Muslim countries in the Middle East impose heavy penalties equivalent to those for opium or cannabis for anyone who carries or uses Khat. The first Islamic country to enforce restriction on Khat was Saudi Arabia; when in 1956, a Royal Decree was issued prohibiting the planting and use of Khat.

In Yemen, the prohibition could not be strictly imposed due to some reasons. According to Laurent[1], the consumption of Khat is less common in Christians, perhaps because they are allowed to consume alcohol. However, some Christians in Harar used Khat and the Bishop of Harar in Ethiopia also considered it as a divine blessing. During the colonial era, a series of laws were enforced in Aden, Djibouti and Somalia to prohibit the use of Khat. However, these legal restrictions proved ineffective and inappropriate and were soon abrogated.

Khat is defined as the leaves and young shoots of *Catha edulis,* a species of the plant, family Celastraceae, which grows wild or is cultivated in Eastern Africa and Southern Arabia and more specifically in Democratic Yemen, Ethiopia, Kenya, Madagaskar, Somalia, Tanzania and Yemen Arab Republic. The inhabitants of these regions are known to chew Khat customarily in order to obtain its stimulant effects. Central stimulation by Khat can manifest itself in euphoria, a feeling of well being, mental alertness, excitement; and enhancement and familiation of associations. The after effects are usually insomnia, numbness and lack of concentration. The continuous and excessive use of Khat can however produce serious side effects. The consumer becomes irritable and quarrelsome, is difficult to handle and antagonistic to all forms of authority. He lives in a dreamworld and becomes mentally divorced from reality and suffers a deterioration of character. He becomes increasingly apathetic, dull in intellect and unable to concentrate;

he is no longer able to work and becomes a burden to his family and friends. The excessive use of Khat may thus create considerable problems of social, health and economic nature. These problems and the current scientific knowledge about Khat has been reviewed in this communication.

HISTORY

It is generally accepted that Khat probably originated and was used in very ancient times in the Ethiopian uplands. A fascinating but not adequately supported hypothesis identifies in Khat the magic smoke that inspired the Delphic pythoness, Holmer's "nepenthe" offered by Helen to Telemacus, and an energetic medicine that Alexander the Great used to cure his army[2].

The first recorded documentation of Khat seems to be manuscript of the first half of the 14th Century by the Sultan of Ifat, Sadar AbDin, in which he states his intention of planting Khat in the enemy city of Marad after its conquest (MS 143, Bibliotheque Nationale, Paris). From the Harar area, Khat was introduced to its present day territories of Somalia, Djibouti, Yemen, Kenya, Madagaskar, Tanzania and down to South Africa. The introduction of Khat to South West Arabia is attributed to Sheikh Abu Zerbin in the year 1424[3].

BOTANY

Khat is known to grow wild mostly on hillsides and mountain slopes at altitudes of 1500-2000m above sea level. The first botanical description of Khat was given by P. Forskal who called it *Catha*. His description was published posthumously in "Flora Aegyptico-Arabica" edited by K. Niebuhr in 1775. The plant has been named *Catha edulis* Forsk in his honour. Khat appears in many varieties and shows a wide range of adaptability. Most taxonomists consider that the genus *Catha* consists of the single species, *Catha edulis* which belongs to Celastraceae family. The

stems, the mid ribs and the prominent veins of Khat vary in a spectra of colours which ranges from green to red. For this reason it is a common practice to distinguish two dominant types of Khat referred to as "white" and "red" Khat[4]. Recently we have described the botanical and pharmacognostical aspects of the Saudi Arabian variant of *Catha edulis*[5].

PATTERN OF KHAT CHEWING

Immediately after picking, Khat branches and sprouts are prepared in bundles tightly wrapped in banana leaves in order to keep them fresh. This procedure is essential since the dried and old leaves lose a great part of their effect probably because some major compound(s) undergo decomposition reaction[6].

Khat chewing has become some kind of social institution. The chewing usually takes place in parties with special patterns where friends gather after work. Parties with men only are more common but sometimes one may witness mixed parties with two or more couples sitting together or the women having a separate session in the same household.

The amount of Khat chewed is variable. It depends on the consumer and the duration of the party. The average amount per person is one bundle of Khat. Only tender leaves and stems are chewed, this makes upto 50 gm of fresh material. The juice is swallowed with the saliva; the residue is not spat out too soon, but gathered in the cheek and kept usually for the whole period of chewing. The bolus thus accumulated makes a characteristic bulge in the cheeks of the chewer. During Khat chewing considerable amounts of liquids (tea and soft drinks) are also ingested. The need for liquids is due to the fact that some active principle of Khat provokes the dryness of mouth.

SOCIAL AND ECONOMIC ASPECTS

The literature on the social and economic effects of Khat use suggests that it contributes to family instability because of the economic drain of the family's resources. In spite of this fact, Khat has become the basis of a lifestyle and plays a dominant role in celebrations, marriages and political meetings. Indeed withdrawal from Khat results in social isolation[7]. Khat chewing has become common both in the urban and in the rural societies of many of the countries known to grow Khat and in their neighbouring countries which import it for their consumption. Even the school and college boys are known to consume large amount of Khat for its stimulant effect.

According to Kervingant[8], the continued use of Khat causes loss of appetite leading to malnutrition. The undernourished system becomes an easy prey to acute and chronic diseases. The consequent rise in morbidity is reflected in an increase in social assistance costs. The consumer's mental faculties are numbed, he loses his will to work, his efficiency becomes low. He loses interest in his family which could be due to his inability to provide the financial support and also to the impotence produced by chronic Khat consumption. The family too may suffer from malnutrition and become liable to contact various infective or deficiency diseases or turn to begging, theft or prostitution in order to earn the livelihood. Finally, there may be a serious economic balance-of-payments problems in those countries where the Khat import accounts for the loss of a sizeable proportion of their national income. The price of Khat on the market follows the law of demand and supply. The average cost of one bundle i.e., about 50 gm of fresh chewable material is around US $ 10 and it may at times rise to 30US $.

MEDICAL ASPECTS AND DEPENDENCE

The consumer gets a feeling of well being and mental alertness with loquacity, excitement and sometimes anxiety[9]. To achieve the

climax of such feelings they continue chewing for four to six or even more hours. The after effects in such people are usually insomnia, numbness, lack of concentration and anorexia. Extensive descriptions are reported by Halbach[9], Hughes[10] and by a WHO advisory group[11]. The above studies point out the following effects: constipation probably due to the astringent effect of tannins, anorexia, stomatitis, œsophagitis, gastritis, meteorism, paralytic ileus, cardiovascular effects such as tachycardia, palpitation sometimes with extrasystoles, hypertension, myocardial insufficiency, cerebral haemorrhage, migraine, hyperthermia, sweating, mydriasis, impairment of sexual activity in man, pulmonary œdema, hepatotoxic effects etc.

The excessive use of Khat induces some degree of psychic dependence[12]. Khat seems, however, not to cause physical dependence or withdrawal syndrome. Aggressive behaviour and toxic psychosis have not been clearly evidenced in Khat users; reactive depression, anxiety and irritation seem to be the most serious psychic effects[10]. The pleasant effects of Khat are a strong inducement for many to procure the necessary supplies often at the expense of vital needs such as food and drugs[12]. Khat chewing habit thus creates the problems of social, health and economic nature.

THE INTERNATIONAL INTEREST AND SCIENTIFIC STUDIES

Before the second world war, the amount of Khat consumed was very limited. Khat chewing spread with the improvement of transportation, liberalization and urbanization. The question of Khat chewing and its undesirable consequences has been raised several times in the international forums since the days of the League of Nations. The 24th Session of the Communication on Narcotic Drugs of the United Nations adopted a resolution recommending research on Khat with special reference to the analysis of active substances, their pharmacological actions and their effects on users from a socio-economic point of view[13].

CHEMISTRY OF KHAT

The chemical studies have revealed that Khat leaves contain a great number of compounds such as alkaloids, glycosides, terpenoids, tannins and flavonoids etc. More than forty alkaloids have been detected in this plant. Many of these are cathedulins of low molecular weight ranging between 600 and 1200[14]. From the point of view of biological activity, the phenylalkylamines are the most important. Cathine ((+) - norpseudoephedrine) was considered until recently to be the main sole active principle of Khat. The cathedulins have not been shown as yet to possess any pharmacological activity.

A new phenylalkylamine compound, not previously reported in nature has recently been found in greater but variable quantities. The chemical structure of this compound has been established and it has been designated as cathinone[15,16]. Cathinone is much more potent stimulant than cathine[16]. It is a highly unstable compound in the presence of oxygen and undergoes decomposition reaction leading to the formation of a dimer[16]. The "red" type of Khat considered superior by users seems to contain cathinone in greater quantities than the white type[4]. Similarly the fresh sample of Khat leaves is also considered to possess greater amount of cathinone as compared to the old dried material.

Apart from the cathedulin and phenylalkylamine alkaloids, tennins and triterpenoids, the studies of El Sissi and Abd Alla[17] have established the presence of some flavonoid compounds including kaempfero, quercetin and myricetin in the fresh leaves of Khat. The recent study of Gellert *et al* [18] has shown the presence of dihydromyricetin and its 3-0-rhamnoside in the fresh leaves. The flavonoids may be responsible for some of the pharmacological actions of Khat[5,19].

PHARMACOLOGICAL ACTIONS

Pharmacological studies have been mostly carried out on the two active principles of Khat cathine and cathinone. The ampheta-

mine like stimulant effect of Khat was initially attributed to cathine[9,20,21,22]. However, this attribution was disputed by reports showing that plant extracts from fresh leaves contained an unknown compound more active than cathine[23]. Recent studies have shown that 1-cathinone is the principal active constituent in the fresh leaf and it rapidly decomposes into the less potent cathine[24]. In this context, it is interesting to note the observations of May *et al*[25] who have shown that dopamine *B*-hydroxylase catalysed ketonization of cathine to cathinone, demonstrated *in vitro*, may also be responsible for its bioactivation to cathinone in the human system and this may account for its pharmacological activity which is qualitatively similar to cathinone and may also explain the delay in the onset of action and a much longer duration of cathine as compared to cathinone. Cathinone has been shown to be approximately 10 times more active than cathine and has an immediate onset of action and a short duration of activity.

Both cathine and cathinone have been shown to possess amphetamine like actions on gross bebaviour, body temperature, locomation, stereotyped and operant behaviour and food intake in the experimental animals. They also enhance electrically stimulated noradrenergic transmission, the mechanism of action of both amines is believed to be the release of transmitter at the end of noradrenergic neurons[11].

Apart from the marked central effects, Al-Meshal *et al*[15] and Tariq *et al*[19] have reported the gastric anti-ulcer activity of Khat and the flavonoidal fraction of its crude extract on Shay rats and against phenylbutazone and reserpine induced ulcers in rats and the histamine induced ulcers in guineapigs. Their results suggest that the gastric anti-ulcer activity may be present in the component(s) of its flavonoid fraction. Further studies in order to pinpoint the exact active principle are under progress.

FOLK MEDICAL USES OF KHAT

Some medicinal employment of Khat has been noted in the countries where it has been used as a stimulant. The most observations have been made by Peters[26]. It could be presumed that most of the mentioned customs are rather out-of-date today.

Somaliland

Khat seems to have a limited medical use among the Somalis. The only uses that have been quoted by Peters are to stimulate urinary activity, and to aid the treatment of genito-urinary diseases, such as retention of urine, and gonorrhea. Besides, there is a belief that the chewing of Khat leaves affords a protection against malaria.

Arabian Peninsula

In the past century it was claimed by informants from some non-specified Arab tribes that a twig of Khat carried in the bossom is a certain safeguard against infections. It was also believed that the land where it grows is secure against the infection of plague (e.g., Bubonic plague). Use is also made of it as an astringent medicine.

Ethiopia

Like the Somalis, the Ethiopians also make a limited use of Khat as a specific medicine. Merab records that dervishes chew small fragments of Khat leaves and spit it on the sick when pronouncing a benediction. Christians as well as Muslims prepare an infusion which they administer to the invalids[2].

Southern Africa

In South Africa an infusion of Khat is used as a remedy for coughs, asthma, and other diseases of the chest[27]. The shoots are also used by the Bushmen as a specially nourishing food. In Tanganyika the leaves are used for influenza, and the roots are also eaten to cure stomach aches[28].

Europe

Also in Europe there has been some interest for introducing this natural drug in medical treatment. It would appear that it could be used, in the first place, as a general stimulant. Various authors have recorded uses for Khat extracts as medicinal substances. Bertherand in 1889 attributed to Khat a great number of possible uses[29]. Peters reported that some pharmacists of Lyon launched a product, prepared from Khat extracts, called Neo Tonique Abyssin in 1910, but the supply soon stopped because of the difficulty in obtaining the raw ingredients at the outbreak of the First World War.

In 1913, a London pharmacist began to manufacture products based on Khat. Dr. Martindale marketed three products: (1) *catha*-cocoa milk, (2) *catha*-cocoa glycerophosphate.... a nervous tonic and stimulant in which milk powder is combined with *catha* extract and calcium glycerophosphate, and (3) effervescent phenolphthalein with *catha*... a mild tonic laxative. Extract of *catha* was also put in tablet form.

Neither the 1948 edition of the British Pharmacopoeia nor the British Pharmaceutical Index of 1949 mention these preparations. However, the Extra Pharmacopoeia lists two preparations under the heading of *Catha edulis* as an adulterant or substitute for tea[30].

Miscellaneous

The Eastern Mediterranean report quotes a recent author who described how a certain magical spirit personified in magical Muslim lore, was regarded as the Patron Spirit of Khat. This spirit comes in aid to women in difficult labour. When invoked, it becomes incarnate and materializes in a human medium. The medium chewing some Khat leaves spits on the genitals of the parturient woman. This, he said, according to their belief, acts as a fire-whip on the spirit producing uterine pain[31].

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A PHARMACOLOGICAL STUDY ON UDESALEEB (PAEONIA EMODI): A UNANI ANTICONVULSANT DRUG

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INDIA

A PHARMACOLOGICAL STUDY ON UDESALEEB (PAEONIA EMODI): A UNANI ANTICONVULSANT DRUG*

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Abstract

The present study was undertaken to evaluate the anti-convulsant and central nervous system depressant activity of aqueous and alcoholic extracts of *Paeonia emodi* Linn. Male albino rats weighing 100-150 gm were subjected to supramaximal electro-shock and pentylenetetrazol induced convulsions. The pretreatment of rats with the aqueous and alcoholic extracts of *Paeonia emodi* Linn. in the dose of 1 gm/100 gm of body weight two hours before the chemically induced seizures showed 17% and 83% protection respectively. Both the aqueous and alcoholic extracts increased the flexion phase in supramaximal electroshock seizures whereas extension phase was reduced by aqueous extract and abolished by alcoholic extract. The results suggest that the alcoholic extract of *Paeonia emodi* Linn. is more potent anticonvulsant as compared to aqueous extract. The extract significantly decreased motor activity of rats suggesting central nervous system depression.

INTRODUCTION

Udesaleeb, a wellknown Arab drug, is the tuber of *Paeonia emodi* Linn[1,2]. It was reported as brain and nerve tonic by Avicenna[3], Lateef[4] and Hussain[5], as the remedy for epilepsy by

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Avicenna[3], Baitar[6], Antaki[7], Kazrooni[8] and Hussain[5], and as tranquilizer by Avicenna[3] and Hussain[5]. However, no scientific data is available to supplement these ancient reports. Hence, the present study has been made to evaluate the anti-convulsant and central nervous system depressant activity of the aqueous and alcohol extract of *Paeonia emodi* Linn.

MATERIAL AND METHODS

Male albino rats, weighing 100-150 gm, were subjected to supramaximal electroshock and pentylenetetrazol induced seizures. Aqueous and alcoholic extracts in the dose of 1 gm/100 gm of body weight were administered orally, one, two and three hours before exposing the animals to electrical shock (1 msec duration, 100/sec, frequency, 140 V amplitude and for total duration of 0.3 sec only) following the method of Toman, *et al* [9]. Duration of clonic and tonic phases of seizures was recorded. Phenobarbitone sodium in the dose of 6 mg/100 gm of body weight was used as the standard drug. Chemical induced convulsions were produced in rats by injecting pentylenetetrazol in the dose of 10 mg/kg of body weight subcutaneously following the method of Toman, *et al.*[10] The aqueous and alcoholic extracts of the drug were given two hours before the pentylenetetrazol injection. The number of animals showing seizures and death within two hours were recorded. Phenobarbitone sodium in the dose of 3 mg/100 gm of body weight was used as the standard drug. The study on the effect of aqueous extract in the dose of 500 mg/ 100 gm of body weight orally on motor activity (spontaneous movements and distance travelled by the animal) of rats was done using an activity cage according to the method of Csanji, *et al*[11]. The movements were recorded for 30 minutes and one hour after the treatment with drugs. Chlorodiazip-

oxide in the dose of 2.5 mg/100 gm of body weight orally was used as standard drug. Effect of aqueous and alcoholic extract in the dose of 1 gm/100 gm of body weight on body temperature was also studied. The rectal temperature of the rats was recorded at 0, 30, 60, 90 and 120 minutes of the administration of extracts.

RESULTS AND DISCUSSION

The effect of aqueous and alcoholic extract of *Paeonia emodi* Linn. in flexion and extension phases of electrically induced convulsions are shown in Table I. The results suggest that *Paeonia emodi* Linn. significantly reduces the extension phase and increases the flexion phase of electrically induced seizures and the peak effect is seen after two hours of treatment with the drug. Phenobarbitone sodium also increases the flexion phase and reduces the extension phase of electrically induced conclusions. Similar pattern was observed in chemically induced shock where both the extracts of *Paeonia emodi* Linn. reduce the incidence of convulsions and death as compared to the untreated group. The aqueous extract showed 17% protection from convulsions and 50% protection from death while the alcoholic extract showed 83% protection from both seizure and death. Phenobarbitone sodium showed 83% protection from convulsions and 100% protection from death. The alcoholic extract is more potent than the aqueous extract in these studies. Our studies on motor activity showed significantly less activity in the rats. The spontaneous movements were reduced from 266.38 ± 26.83 (control group) to 118.75 ± 20.11 (P-.01) and the distance travelled by the test drug treated animal in 30 minutes was reduced to 25.75 ± 4.33 (P - .001) against the normal value of 68.63 ± 6.02 feet in control group. The values in the standard group pre-treated with chlorodiazipoxide were - spontaneous movements 138.25 ± 22.10 (P - .01) and distance

travelled by the animal 43.25 ± 7.07 feet (P-.05). Both the aqueous and alcoholic extracts reduced the body temperature within two hours after the drug administration (Table II). These findings suggest that *Paeonia emodi* Linn. has both anticonvulsant and CNS depressant activity. The results are qualitatively similar to those of phenobarbitone sodium. However, the crude extract is 100-300 times (dose to dose) less potent as compared to phenobarbitone sodium. A separate study was conducted to study the effect of *Paeonia emodi* Linn. on neurotransmitter levels in brain. However, we failed to get any consistent data on the effect of *Paeonia emodi* Linn. on noradrenaline, dopamine and 5-hydroxytryptamine levels in brain. Further studies are required to ascertain the mechanism of action of *Paeonia emodi* Linn.

TABLE I

Effect of aqueous and alcoholic extracts of *Paeonia emodi* Linn. on flexion and extension phases of electrically induced convulsions in rats

Time of drug administration	AQUEOUS EXTRACT		ALCOHLIC EXTRACT		PHENOBARBITONE	
	Flexion Sec.	Extension Sec.	Flexion Sec.	Extension Sec.	Flexion Sec.	Extension Sec.
0 hour	3.50 ± 0.84	11.0 ± 1.41	3.80 ± 0.75	11.0 ± 1.35	3.50 ± 0.83	11.0 ± 1.40
1 hour	4.66 ± 2.30	5.0* ± 1.31	7.0** ± 0.26	0.00*** ± 0.00	11.25* ± 4.57	0.00*** ± 0.00
2 hour	8.50* ± 1.40	2.17* ± 1.28	7.00** ± 0.26	0.00*** ± 0.00	11.30* ± 4.75	0.00*** ± 0.00
3 hour	8.67* ± 1.99	2.50** ± 1.59	6.66* ± 0.91	1.50*** ± 1.02	5.6* ± 0.83	1.33*** ± 0.33

*P - 0.5, **P / 0.01, ***P / 0.001

TABLE II

Effect of aqueous and alcoholic extracts of *Paeonia emodi* Linn. on body temperature in rats

GROUP	Rectal temperature in centigrade				
	0 min	30 min	60 min	90 min	120 min
Control	35.31 ± 0.33	35.31 ± 0.26	35.81 ± 0.38	36.31 ± 0.34	36.31 ± 0.26
Aqueous Extract	35.25 ± 0.67	33.50* ± 0.37	33.50* ± 0.84	32.62* ± 0.78	32.62* ± 0.82
Alcoholic Extract	37.38 ± 0.22	36.06*** ± 0.44	35.38*** ± 0.35	34.88*** ± 0.28	34.38*** ± 0.26

NOTE:

P is calculated in comparison to the temperature before administration of the drug.
*P / 0.5, ***P / .001

SUMMARY

A study on the anti-convulsant and CNS decressant activity of aqueous and alcoholic extract of *Paeonia emodi* Linn. in rats reveals that both the extracts increase the flexion phase and significantly reduce the extension phase in electrically induced convulsions, reduce the incidence of convulsions and death in chemically induced shock and reduce the body temperature and the motor activity. The acoholic extract was found to be more potent than the aqueous extract. These findings suggest that *Paeonia emodi* Linn. has both anticonvulsant and CNS depressant activity.

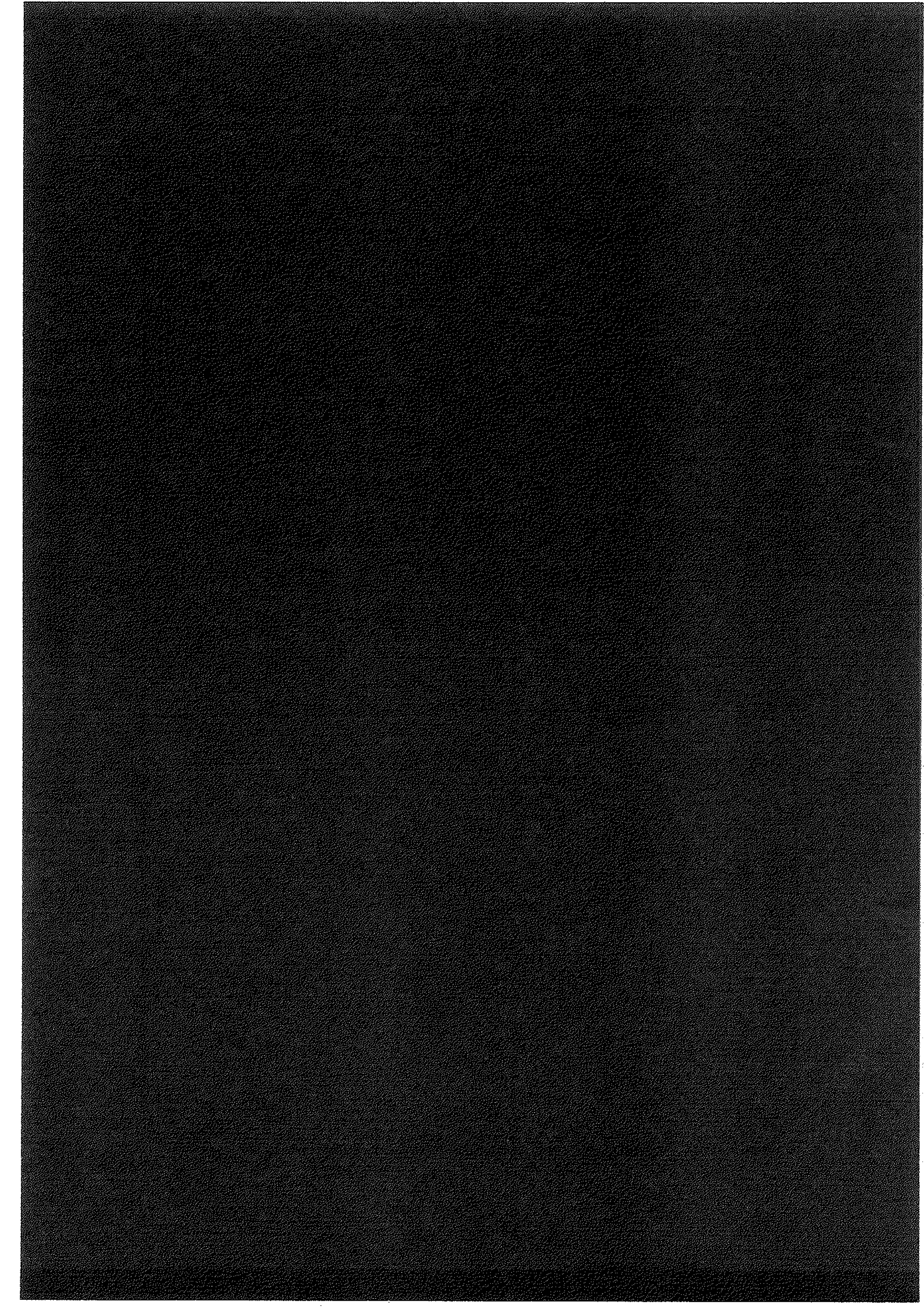
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STUDIES ON HYPOGLYCEMIC ACTIVITY OF POTERIUM SPINOSUM

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STUDIES ON HYPOGLYCEMIC ACTIVITY OF POTERIUM SPINOSUM*

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INTRODUCTION

Poterium spinosum is reputed for its antidiabetic properties in common man of Jordan. The use of this plant in these populations suggests the need for experimental assessment concerning its hypoglycemic effect in laboratory animal, as well as in induced diabetic conditions. *Poterium spinosum* is reported in "Flora Europea"[1] as sarcopoterium in family Rosaceae. The presence of antidiabetic properties in roots and other parts of plant does not seem to be reported earlier, hence the present study was undertaken to investigate the hypoglycemic activity of *Poterium spinosum* extract on administration in normal and alloxanised rats and suggest the possible mechanism of action.

MATERIAL AND METHODS

Poterium spinosum was collected during the Summer season in Jordan. It was air dried and washed of any dust. Whole roots were chopped to fine pieces and 100 gm were immersed in 500 ml of distilled water for 30 minutes. It was then boiled for 30 minutes so that the volume of water remained approximately one third of its original volume. Then the extract was filtered. The filtrate was brick red in colour and was further concentrated to 125 ml. Solid content was found to be 2.37 per cent on dried root basis in the extract. On

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qualitative analysis, the extract gave positive tests for the flavonoid, tannin and saponin while it was negative for alkaloids.

Male albino rats weighing between 200-300 gm of Wistar Sprague Dawley strain obtained from the animal colony of Jinnah Postgraduate Medical Centre, Karachi, were used throughout this study. They were kept in the animal house at about 27°C. In case death occured in the diabetic animals, the number was made up from a duplicate group of the same date.

Alloxan monohydrate (obtained from Sigma Chemical Co., USA) was prepared as 5 per cent solution in double distilled water, and 200mg/kg body weight was injected in animals fasted overnight for 14-18 hours[2]. Food and water was supplied immediately after injection. *Poterium spinosum* water extract and phenformin hydrochloride solution in water were administered through a stomach tube fitted to a syringe. The dose of *Poterium spinosum* and phenformin hydrochloride was expressed in terms of solid present in the extract and the solution of phenformin hydrochloride.

Blood samples were collected as required from tail or by direct heart puncture. The animals were fasted for 12 to 16 hours before they were sacrificed. The animals were stunned by a blow over the head, blood was collected directly from the heart, inserting heparinized syringe. The blood was pooled from each group for lactate and pyruvate determination. Rats were killed by dislocation of the neck. The liver was rapidly removed and pressed between metal clamps previously cooled in liquid nitrogen. The time elapsed between dislocation of the neck and deep freezing the tissue was not more than 10 seconds. The frozen liver was pulverized in a mortar to a fine powder with frequent addition of liquid nitrogen. The powder was transferred to a weighed plastic centrifuge tube containing 2ml of frozen 30% (w/v) perchloric acid. After a rapid reweighing, the tissue (1-2 gm) was mixed with perchloric acid, care being taken that no thawing occured. Ice cold 5ml distilled water

was added and the mixture was immediately homogenized in the centrifuge tube with a pestle driven by a low speed motor. This was continued for about 2 minutes until thawing was complete. Protein was removed by centrifugation in a refrigerated centrifuge at 1000x gm for 10 minutes. The supernatant fluid was adjusted to pH 5-6 with 20% (w/v) potassium hydroxide and after standing for 30 minutes in cold, the precipitate was centrifuged. The supernatant fluid was then shaken for 30 seconds with 0.1 gm/ml florisil 60 100 mesh (obtained from Merck, Darmstadt, W. Germany). This treatment removed flavins from the solution and decreased the slow non enzymatic oxidation of NADH, while the recovery of the metabolites determined was not affected. The florisil was removed by centrifugation and the supernatant fluid was used for the analysis.

Glucose was determined by the method of Nelson Somogyi[3], enzymatic assays were performed for lactate and pyruvate by Sigma Kit[4] and triglyceride was determined by Sigma Kit[5].

RESULTS

The alloxan diabetic rats were treated with 5, 10, 50 and 100mg of the extract and normal rats with 100mg of the extract. The maximum hypoglycemia was observed in all the alloxanized diabetic rats after 3 hours of treatment with *Poterium spinosum*. The group which was administered oral dose of 10mg body weight of *Poterium spinosum* extract showed the maximum hypoglycemia (60% blood glucose decrease as compared with zero hour) after 3 hours, when compared with other groups given different doses. Thus 10mg/kg body weight was used in further experiments.

Effect of *Poterium spinosum* on blood metabolite concentrations is shown in Table I. A significant increase was observed in diabetic rats in glucose, lactate and triglyceride levels as compared with control rats. When *Poterium spinosum* was administered to

the diabetic rats, blood glucose level was significantly decreased while lactate, pyruvate and triglyceride were increased as compared with diabetic control group. The treatment of diabetic rats with phenformin hydrochloride showed similar effect as the *Poterium spinosum*. There was a significant increase in glucose and lactate levels of diabetic rats as compared with that of normal control rats (Table I). In diabetic rats 3 hours after treatment with *Poterium spinosum*, a significant decrease in hepatic glucose concentration was observed when compared with diabetic control. Treatment with phenformin hydrochloride did not show any significant effect on hepatic metabolite concentrations in diabetic rats as compared with diabetic control.

DISCUSSION

Apart from effects on hormonal status or hormonal responsiveness of tissues, pharmacological hypoglycemia may be produced by the three main metabolic actions: stimulation of glucose uptake and utilization by tissue, inhibition of glucose production by liver, or inhibition of the supply of glucogenic precursors by peripheral tissues[6].

In our study, glucose level decreased in both liver and blood and there was an increase in blood lactate (Table I) which may partly be due to increased peripheral glucose utilization and increased glycolysis or partial inhibition of hepatic gluconeogenesis. The hypoglycemia may not be due to diminished release of gluconeogenic precursor by peripheral tissues because blood lactate and pyruvate level were increased (Table I). Reduced level of serum triglyceride in diabetic rats treated with *Poterium spinosum* may be due to the oxidation of free fatty acids rather than due to esterification. The possible mechanism of action of *Poterium spinosum* may be either through increased glucose uptake and glycolysis with increased lactate production from periphery or by

partial inhibition of hepatic glyconeogenesis, which is also observed in phenformin hydrochloride treatment.

As *Poterium spinosum* extract was not found to be effective in normal rats but was effective in alloxan diabetic rats, its action is not dependent on insulin production or insulin protection. In alloxan diabetic rats the insulin is decreased and therefore any fall in blood sugar by a hypoglycemic agent would be by its direct action or by helping in some way the action of poorly available endogenous insulin. The action of *Poterium spinosum* is comparable to that of phenformin (Table I).

Phenformin and other biguanides do not affect insulin secretion but increase glucose utilization apparently because they inhibit oxidative metabolism of glucose and consequently increase anaerobic glycolysis within the cells. They also decrease glucose absorption.

The formation of insulin tannin complexes have been shown to be biologically active. Three flavonoid compounds were isolated from *Ficus bengalensis*, which possessed hypoglycemic activity[7]. It is conceivable that probably *Poterium spinosum* contains some flavonoid and tannin compounds, which exert hypoglycemic action by complex formation with endogenous insulin. From the results it is concluded that *Poterium spinosum* is a potent oral antidiabetic agent and the exact mechanism of action is not known which could be useful for oral treatment of diabetes.

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TABLE I

Effect of treatment of *Poterium spinosum* Extract and Phenformin on Body and Liver weights and Blood and Liver Glucose, Lactate and Pyruvate.

The values are expressed as mean ± s.e.m. The number of observations is given in parentheses.

	Body weight (gm)	Glucose		Lactate		Pyruvate		Triglycerides	
		Liver weight (gm)	Blood (mmole/L)	Liver umole/g)	Blood mmole/L)	Liver mmole/g)	Blood mmole/L)	Liver umole/g)	Blood (mg/dl)
Normal Control	254.0 ±9.0 (10)	5.3 ±0.2 (5)	4.1 ±0.3 (5)	6.5 ±0.7 (5)	1.6 ±0.1 (5)	1.5 ±0.2 (5)	0.05 ±0.004 (5)	0.02 ±0.002 (5)	30.0 ±4.8 (5)
Diabetic Control	227.7 ±9.8 (10)	5.7 ±0.1 (5)	20.1° ±2.0 (5)	19.7° ±1.3 (5)	2.6+ ±0.2 (5)	2.6x ±0.2 (5)	0.05 ±0.003 (5)	0.03 ±0.007 (5)	332.0° ±24.0 (5)
Diabetic rats treated with *Poterium spinosum* (10mg/Kg)	229.0 ±7.7 (10)	5.6 ±0.1 (5)	10.4** ±0.5 (5)	8.2** ±0.9 (5)	3.8** ±0.3 (5)	3.7 ±0.5 (5)	0.1** ±0.003 (5)	0.04 ±0.007 (5)	260.2* ±16.4 (5)
Diabetic rats treated with Phenformin (100 mg/Kg)	228.0 ±9.5 (10)	5.4 ±0.1 (5)	14.3* ±0.9 (5)	17.0 ±0.5 (5)	3.9*** ±0.2 (5)	3.6 ±0.5 (5)	0.1** ±0.002 (5)	0.06 ±0.01 (5)	311.8 ±34.7 (5)

*P < 0.05, **P < 0.01 ***P < 0.001, when compared with diabetic control
x P < 0.05; + P < 0.01; o P < 0.001, when compared with normal control

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A MODEL SCIENTIFIC RESEARCH ON A DRUG OF ISLAMIC MEDICINE: HYPOCHOLESTEROLEMIC EFFECT OF ALLIUM SATIVUM AND ITS POTENTIAL PROTECTIVE ACTION AGAINST CORONARY HEART DISEASE

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A MODEL SCIENTIFIC RESEARCH ON A DRUG OF ISLAMIC MEDICINE: HYPOCHOLESTEROLEMIC EFFECT OF ALLIUM SATIVUM AND ITS POTENTIAL PROTECTIVE ACTION AGAINST CORONARY HEART DISEASE

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INTRODUCTION

For centuries people have revered the extraordinary medicinal properties of garlic (*Allium sativum*, Linn). The Babylonians used it to treat diseases as early as 3000 B.C. In Egypt, garlic was believed to give slaves the strength to endure hard labour and to give soldiers courage in the battle field. After intense fatigue a clove of garlic slowly chewed, and swallowed, has been reported to act as a very powerful restorative.

In Islamic Medicine, too, garlic has been extensively used, and prescribed. In 1893 Dymok summarised the old literature on garlic. Under its history and use, he records, among other things, "Garlic is the *Thum* of the Arabians and *Seer* of the Persian.... A decoction of garlic in milk is given in small doses in hysteria, flatulence, sciatica, and heart disease...." These claims in the past have been made on the basis of personal experiences of old physicians. However, during the last few decades hypocholesterolemic properties of garlic have been widely reported. The magnitude of this action ranges form a modest 14% lowering of serum cholesterol in humans to a lowering of 80% in cholesterol-fed hypercholesterolemic rabbits. Garlic was fed in these various studies as the raw

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cloves, steamed or fried cloves, as garlic (oil) pearls, powder, as juice or extract or as the essential oil.

In most of the studies made so far it has been assumed that hyocholesterolemic activity in garlic resides only in the very small quantity (about 0.25%) of the (essential) oil, which is responsible for the flavour and taste of the herb. In 1944, C.J. Cavallito and J.H. Bailey isolated this oil, which in its pure form is pale yellow to colourless, is of a very repulsive taste and has the true concentrated odour of garlic. They succeeded in isolating from it 'allicin' a sulphur compound, and determined its structure as diallyl disul-phide-oxide (I). An amino acid allicin is also present in garlic. Its structure has been determined to be S-allycysteine sulphoxide (II). In the intact clove 'alliin' and the enzyme allinase are kept apart by the cell walls. However, when the clove is cut the enzyme allinase reacts with alliin and converts it into allicin, which has the characteristic galric odour.

$$\underset{\text{I}}{CH_2 = CH.CH_2.\overset{\overset{O}{|}}{S}CH_2.\overset{\overset{NH_2}{|}}{C}HCO_2H} \xrightarrow{\text{Allinase}} \underset{\text{II}}{\begin{matrix} CH_2 = CH.CH_2.S \\ CH_2 = CH.CH_2.S \longrightarrow O \end{matrix}}$$

Alliin (S-allylcysteine sulfoxide) **Allicin (diallyl disulfide-oxide)**

The effects of maturation and of the postharvest changes in garlic on this hypocholesterolemic property have not been studied. Such effects might explain some of the inconsistencies in the results of various studies of different garlic products. Alliin and allicin, two pure products isolable from garlic have been shown to lower cholesterol levels of cholesterol-fed and normal rats. But this activity does not account for the total activity of the whole garlic paste. Besides, according to recent Japanese work*, allicin taken in

* Personal Communication (Prof. Kitabara, S. Kunamoto University).

large quantities can destroy the membranes of the red blood cells, and inflame the gastro-intestinal tract. These reports prompted us to look for hypocholestrolemic activity of garlic in its various extractives in non-polar, to progressively more polar solvents, and to confirm this activity through a study of their action on various enzymes, which have been well studied and well established for the synthesis of cholesterol in the body.

The previous studies have been mostly made using rabbit or rat as the model animal. The overall effects of garlic and its constituents on lipid metabolism in chicken have not been described. Work done on rats and rabbits is of value, but chicken (or quail) is a better model to study atherosclerotic changes, because the lipoproteins of chicken (or quail) are more closely related to those of the humans.

In this study we describe the effects of feeding garlic or garlic extracts on hepatic 3-hydroxy-3-methyl glutrayl-cœnzyme A (HMG-CoA) reductase, cholesterol 7α-hyroxylase, cholesterol 7α-hydroxylase pentose-phosphate pathway enzymes and fatty acid synthetase (FAS) activities. These enzymatic activities reflecting the control of overall lipid metabolism were measured, along with serum lipid components: total cholesterol, LDL-chol (low and very low density lipoprotein bound cholesterol), HDL-chol (high density lipoprotein bound cholesterol),and tryglycerides, in layer and broiler pullets after a fasting-refeeding regimen that induced lipogenic activity.

MATERIALS AND METHODS

Materials: Experimental materials and enzyme reagents were obtained from renowned suppliers. Cholesterol was recrystallised fresh twice from glacialacetic acid, and dried in vacuum. Steam-distilled commercial garlic oil was of J. Manheimer, Inc., Long Island, NY. Garlic bulbs and the diet components were purchased locally. All other chemicals were of analytical grade.

Pullets, diets, experimental design: White Leghorn pullets (layers) 33-37 days of age and the crossbred broiler pullets (broilers) 21 days of age were purchased from a local hatchery. The pullets were randomly distributed into groups (8 layers; 6 broilers), which were placed in a threetier battery. Commercial grower mashes formulated for layers and broilers were fed prior to the start of the experiments. At 8 weeks of age, pullets in each group were weighed and returned to the battery. The experimental diets were based on commercial formulations. The layer diet contained 78% corn, 16% soybean meal (44% protein), 2% alfalfa meal (17% protein) and 1.5% meat and bone meal. The broiler diet consisted of 70% corn, 23.5% soybean meal (44% protein), 2% alfalfa meal (17% protein) and 2% meat and bone meal. Mineral and vitamin supplements are given as footnotes to Tables 1 and 2. The experimental diets were modified with the substitution of 3.8% garlic paste or a solvent fraction or garlic oil equivalent to 3.8% garlic paste for an equal quantity of corn. The groups of pullets, which had been established 2 or 4 weeks prior to the experiment, were randomly assigned the experimental diets. The layers were fed the diets *ad libitum* for 24 days. The control group of broilers was fed *ad libitum* for 24 days; the experimental broiler groups were fed isogravically with the control. Feed consumption of the broilers was recorded. Water was given *ad libitum*. At the end of the 24-day feeding trial, the pullets were fasted for 24 hours and then refed for 72 hours. Blood samples were taken from wing veins of the broilers at the end of the fasting period. After refeeding, the pullets were weighed, killed by severing the carotid arteries and blood samples were collected. The livers were removed, washed, chilled on ice, weighed and then prepared for analyses.

Preparation of the garlic paste and solvent fractionations: Garlic bulbs (1 kg) with the outer and inner husks removed yielded 760g cloves. The cleaned cloves were homogenized to a paste with a commercial Waring blender. One-half of the galric paste was mixed with sufficient corn to provide 7800g of mixture for the formulation of the "garlic paste" experimental diet for the layers or 7000g for the broilers. The remainder of the paste was stirred with light petroleum

ether (500 ml) for 2 hours. After standing for 1 hour, the solvent was decanted. The procedure was repeated twice. The last extraction was completed by filtering the solvent through a sintered glass funnel under vacuum. The three extracts were combined and then concentrated to dryness under vacuum at 60°. The semisolid garlic residue was lyophilized, and the resulting powder was extracted successively as described above with methyl alcohol and water. The final residue was dried overnight in an oven at 60°. The fractionation yielded 1.4g (1.1%) petroleum ether-soluble fraction (PESF), 34.2g (26.4%) methyl alcohol-soluble fraction (MESF), 79.3g (62.4%) water-soluble fraction (WASF) and 12.1g (9.5%) of residue. The values in parentheses correspond to the per cent dry weight in each fraction. Each fraction was taken up in a minimal volume of the appropriate solvent and added to sufficient corn to give 7800g or 7000g. The experimental diets thus contained the specific solubles in quantities equivalent to those in a 5% garlic bulb (3.8% garlic paste) diet. The solvent was removed from each diet by air-drying the mixture spread thinly in a pan overnight in a fume hood.

Preparation of liver homogenates and assays of enzymes: The liver homogenates were preared in 0.1M potassium phosphate buffer, pH 7.4, containing 4 mM $MgCl_2$, 1 mM EDTA, and 2 mM dithothreitol. The tissue was chopped and suspended in the buffer (1:2, wt: vol), and homogenization was done at 0-4° with a Polytron homogenizer. The 100,000 x g supernates (cytosols) and precipitates (microsomes) were stored at -20° prior to assay for enzymatic activities.

Assays for HMG-CoA reductase and cholesterol 7α-hydroxylase was done by standard proldures. The activities of glucose-6-phosphate dehydrogenase, 6-phospho-gluconate dehydrogenase, malic enzyme, citrate-cleavage enzyme and fatty acid synthetase in the cytosol were assayed spectrophotometrically. Protein concentrations were estimated by a modification of the biuret method with bovine serum albumin as a standard.

Estimation of serum cholesterol and triglyceride concentrations: Cholesterol and triglyceride concentrations in serum samples were estimated by using Worthington "Cholesterol Reagent" and "Triglyceride Reagent" kits.

Low density (LDL) and very low density (VLDL) lipoproteins were isolated from the serum (100µl) by precipitation with a mixture of phosphotungstic acid, 9.7 mM (10µl) + $MgCl_2$ 0.4 M (10µl). After standing for 5 minutes at room temperature, the mixtures were centrifuged at 2000 xg for 10 minutes, the supernatent was removed and used to determine the level of cholesterol in high density lipoprotein (HDL). The precipitate was dissolved in 0.1M sodium citrate (100µl), and the level of cholesterol (LDL + VLDL) was estimated by using the above method. This measure was used to verify the accuracy of the estimates of LDL-chol which were calculated from (totalchol) - (HDL-chol + triglycerides).

Expression of data and statistical methods: Enzyme data are presented as specific activities units/(miligram cytosolic or microsmal protein/ minute). Statistical comparison of results was performed by a one-way analysis of variance. When the F test indicated a significant effect, the differences between the means were analyzed by a protected least squares difference test.

RESULTS

The average weight gain of the groups of layers fed the experimental diets (384.0 ± 13.3g) was equal to that of the control diet group (381 ± 31g; Table 1). Although feed consumption was not monitored in this experiment, the above data suggest that the incorporation of the garlic products into the diets did not decrease feed consumption. Significant treatment effects were shown for all aspects of cholesterol and lipid metabolism save that of the HDL-chol concentrations (Tables 3,4). HMG-CoA reductase activity in all experimental groups except the group fed the garlic residue

exhibited less than 30% of the control activity ($P<0.01$). The solvent extracts - PESF, MESF and WASF - were the more potent sources of the HMG-CoA reductase inhibitor ($P < 0.01$) compared to garlic paste and garlic oil (Table 3). A similar but less pronounced pattern of inhibition was found for cholesterol 7 α-hydroxylase (MESF, 49%), fatty acid synthetase (PESF, 71%), malic enzyme (MESF, 73%), glucose 6-phosphte dehydrogenase (MESF, 61%) and 6-phosphogluconate dehydrogenase (WASF, 68%). In the parentheses are shown the most effective treatment and enzyme activity as percentage of control (Table 3).

The above pattern of enzyme inhibition by the various garlic factors was expressed in the serum levels of cholesterol and triglycerides (Table 4). All garlic products except the residue lowered the serum cholesterol level, specifically the LDL-chol fraction. The MESF was most potent, lowering cholesterol and LDL-chol by 25 and 41%, respectively. HDL-chol was not affected by the treatments. These lowerings of the total cholesterol and LDL-chol are reflected in the ratios of total cholesterol: HDL-chol and LDL-chol: HDL-chol shown in Table 4.

A similar experiment was carried out by using pullets of a commercial broiler strain bred for high rates of gain and feed efficiency. The basal diet fed to the broilers contained 23.5% soybean meal (44%) and 70% corn (Table 2). Garlic (3.8%) or the 3.8% equivalent garlic extract was added to the diet at the expense of corn. Feed consumption (18.84 ± 0.17 kg) did not vary between groups; the control group gained an average of 755g, a 53% increase in body weight. The group fed the WASF gained 828g, a 52% increase in body weight. Gains by the groups fed PESF and MESF were equal to that of the control group, whereas groups fed the garlic residue and garlic oil gained 667 and 683g, respectively increases of 43% in body weight. The major deviation in body weight gain was recorded for the group fed garlic paste. The average

weight gain of this group was 632g, only a 40% increase in body weight. Feed consumption by this group was 18.60 kg; whether or not the 3.8% dilution in the energy density of this diet influenced this weight performance could not be determined.

The results shown in Table 5 confirm that factors extracted from garlic suppress hepatic HMG-CoA reductase. Feeding the MESF and WASF of garlic reduced this activity by 66-68%. HMG-CoA reductase was lowered by 50% or more by each of the remaining galric treatments except that of the extracted residue. These suppressions of HMG-CoA reductase are reflected in cholesterol levels of serum taken from fasted and from refed pullets and in the LDL-chol levels of refed birds (Table 5). The MESF, the most potent source of this suppressor, lowered total cholesterol by 25% and LDL-chol by 43%. HDL-chol was not affected by the cholesterol-suppressive agents of garlic. Compared to the control, fatty acid synthetase activity was significantly lower in each of the experimental groups except that fed the extracted residue. The MESF and WASF of garlic were the more potent suppressors of this activity (Table 6). This impact of galric on fatty acid biosynthesis was also manifested in the inhibition of two pentose-phosphate path enzymes, malic enzyme and citrate cleavage enzyme (Table 6). Glucose-6-phosphate dehydrogenase and 6-phospho-gluconate dehydrogenase activities were low compared to fatty acid synthetase and malic enzyme activities. The low pentosephosphate pathway activities in the avian liver (Tales 2,4) have also been reported by others.

In general, the impact of garlic and of soluble fractions of garlic on lipid metabolism in layers was confirmed by using a broiler strain of chicks. All garlic treatments except that involving the extracted residue were associated generally with reduced lipogenic cholesterolopoietic activities and with lower serum total cholesterol and LDL-chol levels.

DISCUSSION

Serum cholesterol levels fall when chickens as well as other species are fed garlic-supplemented diets. Neither the mechanism of the hypocholesterolemic action nor its agent has been clearly defined. When fed in conjunction with cholesterol, garlic decreased the incorporation of acetate into cholesterol and increased fecal bile acid and neutral sterol excretion. Both actions are consistent with the lowering of serum cholesterol. Anomalously, the activity of HMG-CoA reductase was increased and that of cholesterol 7 α-hydroxylase decreased; both responses would be consistent, in cholesterol-fed animals, with the elevation of serum cholesterol.

In chickens fed diets essentially free of cholesterol, the suppressive action of garlic is clearly at the level of cholesterol biosynthesis. The suppression of HMG-CoA reductase is manifested in the decreased serum concentrations of LDL-chol and total cholesterol. The garlic-mediated change in the LDL-chol: HDL-chol ratio of the pullets (-30%) is very similar to the change reported in the cholesterol-fed rat.

Addition of steam-distilled garlic oil, a product widely reported to be a cholesterol-suppressive agent, at a level (0.014%) equivalent to the addition of 3.8% garlic paste elicited similar responses.

The suppressor, readily exctracted from garlic paste with the sequential application of solvents of increasing polarity, was not sharply defined in terms of polarity. The broad distribution suggests either the presence in garlic of polar and nonpolar mediators of cholesterol biosynthesis or alternatively the presence of an active substance conjugated in various forms to the extent that it is distributed among the solvents employed. An inhibitor of fatty acid oxygenases, present in onion and garlic and in steam-distilled garlic oil, exhibits a similar lack of definition in terms of polarity. Alliin and allicin, the highly polar, odorous compounds in garlic, reported to lower plasma cholesterol, are readily extracted in the methanol step of the fractionation sequence. These compounds

conjugated in less polar form could be the active agents in the PESF. However, the demonstration that the odourless WASF is as effective as the MESF in supressing HMG-CoA reductase implies that compounds other than those previously identified are involved in the garlic-mediated suppression of cholesterol biosynthesis.

Key lipogenic enzymes were suppressed to varying degrees by the garlic additives. Differences in the potency of the lipogenic suppressor in each of the solvent fractions were recorded. This discrimination was not expressed towards HMG-CoA reductase, perhaps due to the presence of sufficient suppressor in each solvent extract to dampen this enzyme to some residual level of activity, a level 35% of control in broilers and 25% of control in layers. In layers, HMG-CoA reductase, cholesterol 7 α-hydroxylase, total serum cholesterol and serum LDL-chol values were each clustered about two points, a high point for control and garlic residues treatments and a low point for the five garlic treatments.

The above study establishes the cholesterol lowering properties, on modern scientific lines, of an Islamic herbal medicine for which in the past claims had been made to be effective in heart disease purely on the basis of experience and tradition only.

Cholesterol is a normal constituent of the body. Its daily turn over in an average human body is about 200 mg, a good part of which is produced endogenously (mainly by the liver). Thus in a life span of 70 years, the body produces about 50 kg of colesterol. Its major concentration in the body is in the brain, spinal cord, liver, and blood. It forms an integral of cell membranes. It is the precursor of bile acids, adrenal and sex hormones. It is therefore difficult to imagine that this endogenous cholesterol should heavily contribute to the destruction of our cardiovascular systems. It has been reported that in a variety of pathological conditions, in combination with calcium, fibrin, collagen etc., it forms a large part of the lesion (scars, tubercles, gumata old fibroids, thrombi, cholesteatomata), and performs a repair function. Thus it is

possible cholesterol starts accumulating as a consequence of the lesion and not as its cause. This idea finds strength from recent findings that cholesterol even if briefly exposed to atmosphere picks oxygen, resulting in oxygenated products, the presence of which in micro quantities can only be demonstrated by extremely sensitive techniques developed very recently. Further it has been shown that hydroxycholesterol when consumed even in pico-gram quantities causes lesions in aorta of rabbit.

We have recently started a project to look:

i - How garlic (or its fractions) can regulate the delicate balance between LDL and HDL (the serum lipoproteins) in the body, as LDL is responsible to provide cholesterol through its circulation whenever needed in the body, whereas HDL directs it to liver where it is converted into bile acids and is excreted.

ii - Whether garlic (and its fractions) can prevent formation of lesions, or reverse the process where lesions have already been formed on account of the intake of oxygenated products of cholesterol (or oxygenated lipids in the process of prolonged frying of foods).

This study, we hope, will place in our hands an effective natural dietary product to counter arteriosclerosis in humans. The serum cholesterol-lowering synthetic drugs (triparanol, estrogens, clofibrate etc.) at present in use in modern medicine, have serious side-effects, and their prolonged use turns out to be harmful.

The Reasons for Decline in Research on Traditional Medicinal Plant Drugs: Until the beginning of the current century most of drugs in use were of vegetable, mineral, or animal origin. Most of them had to be procured from the tropical areas of the world. With the advent of Organic Chemistry in Europe a good deal became to know about the chemical structure of the pure compounds which could be relatively easily isolated and luckily also turned out to be the active principles (active in much smaller dosage) responsible for the major total effect of the bulky herbal drug. This gave fillip to the synthesis of drugs - using structures of the active principles as

templates - from simple and locally available raw materials to the coal and petroleum distillation industries. This also obviated the dependance on tropics for costly herbal drug materials, and long wait involved in their import.

The synthesis made possible the production of drugs on a much larger scale in a factory, and at a much cheaper cost. Simple screening and quality control procedures were soon evolved, and even some very simple structure molecules proved to be equally effective. The number of drugs started multiplying and it gave rise to a very profitable, and profit-oriented pharmaceutical industry. This way very powerful, and fast-acting tools became available to the medical profession. But in this process both medical profession and pharmaceutical industry relegated from a noble human service to a purely materialistic business.

However, within a few decades of the use of synthetic drugs, many started showing very serious side-effects - a few were even irreversible resulting in fatalities. This necessitated strict government controls in Europe, and more so in America. Today to put a new research drug on the market by a pharmaceutical company in the U.S.A., it has been estimated, it requires an expenditure of nearly 60 million dollars, and a time lag of 3-7 years to get the final FDA approval. After this huge expenditure (research + FDA + manufacturing/packaging/marketing/promotional and patent coverage costs) the firm is in hurry to recover costs and to make profits, as inspite of all the precautions, the drug is likely to manifest side-effects, or face a competition from a better or faster acting drug within a decade or even earlier. The pharmaceutical firms in the industrialized countries are therefore reluctant to take up research on herbal drugs, as they may be safe but are usually slower acting and require huge expenditure enumerated above, and are likely to take longer to get FDA approval.

There is far too great a stress on animal trials even in the case of innocous dietary herbals for their investigation to regulate some

body processes, first for the fear that humans may not be used as guinea-pigs, although humanbeings have been using these herbals in their diet for the last many centuries, without suffering any ill effects. On the other hand when very potent synthetic drugs are withdrawn on showing side-effects, have the human beings been not used as guinea-pigs in the intervening periods?

A few of the drugs, which have been withdrawn, or put on very restricted careful use in developed countries, are still being marketted by multinationals in developing countries, just to reap fat profits.

In this whole process, the worst sufferers are the developing countries, which are dependent on industrialized countries for drugs, or drug raw material supplies at a cost which they cannot afford, and at the same time they have ceased to investigate their own herbal drugs, which at least could be developed within the countries, and after establishment of their activity on modern scientific lines, could possibly be used in the vast rural areas where they could be grown.

What has been Wrong in Research on Herbal Drugs in the Developing Countries: In doing research on medicinal plants in developing countries, we have been copying the example of Europe and America. Instead of establishing the clinical activity/efficacy for which the drug had the reputation of curing a disease, we in most cases have been resorting to extraction procedures, whereby we succeed in isolating some cyrstalline product(s), and call it "Active Principle"; determine its structure with the powerful tools now available, and are content with publications on novel and complicated structures only. In a still fewer cases these crystalline (or pure) products are subjected to general pharmaccological screening and finding no or very poor activity, are declared the herbal drug of no use.

It is quite possible that in the extraction process we may have missed the actual "Active Principle".

Probably for the same reasons, a few of huge programmes at a cost of billions of dollars at N.I.H. (U.S.A.) and similar institutes in Europe or elsewhere on medicinal plants to discover curative agents against some of the killer diseases (like cancer and heart disease etc.) have met with very little success, and have been dropped.

What Can or Should Now be Done?: These days traditional doctors lack the modern knowledge, and assistance of available sophisticated techniques for diagnosis of disease, and facilities for research on drugs. They rely on their imperical visual observations, and on descriptions of the use of herbal drugs for the treatment of disease developed a few centuries back. These descriptions, no doubt, are the sum total of personal experiences spread over many centuries. Some of the descriptions may have been distorted through translations. However, if properly investigated, they may be proved to be treasure houses worth rediscovery.

In a W.H.O. report it has been observed that chances of clinical verification of claims of herbal drugs are almost 50:50 if investigations are made in collaboration with traditional healers. In Asia and Africa where traditional medicine is still practiced and people go to these healers of their own free will suitable observation teams comprising of traditional healer, clinicians, and scientists should work together to provide modern aids in diagnosis of disease, and the course of disease and cure should be monitored together. The observations should be made with a sympathetic rather than the common preconceived antagonistic attitude. Then the observations showing definite promise should be repeated by other teams and on larger number of patients, taking care that botanically the herbs used are properly and correctly identified and recorded. For the curative herbs so discovered quality control procedures should be developed and these standardized products could be used in health-care programmes, at least in the developing countries. For further study extractions may be made carefully monitoring where the activity is going. Most active fractions

then should be investigated in detail for isolation of the active principles, and determination of their structures.

The start should be made with herbal drugs which are dietary, and hence through their long use have proved to be nontoxic, and hence there should be no hesitation in their trials directly on humans as is being proposed to be done in establishing the hypocholestrolemic properties of garlic in the treatment of arterio-sclerosis in an extension of the above reported study.

With the growing cost of health-care - almost becoming unbearable for the common man - and manifestation of serious side-effects on prolonged use of some of the synthetics, there is already a growing tendency even in America, Europe, and other developed countries to go back to the natural drugs, which is particularly manifest from a very rapid growth in healthfood stores there. Many of the herbs are being sold and used as health-foods to avoid long, rigorous, cumbersome, and costly FDA approvals, which certainly are unnecessary, at least, in the case of dietary herbs.

Logically, to correct the (natural) disorders of the human body, one should look to the natural diets (including herbs) with which man has interacted for centuries, and has accumulated the imperical knowledge. The scientists (including medical scientists) should get together at the sites where such herbals grow and are used, first to confirm on modern scientific lines, the imperical knowledge which exists there, then record it and standardize for common use. It may then be extended for extractions, isolations, structure determination, and study of the mechanisms of the observed actions. Through such an approach, I am sure, we shall soon discover many effective and safe medicinal agents even for the so far unconquered killer diseases. Besides affordable health-care, programmes, particularly for the poorer nations, can be developed. In the past we have done the process in the reverse order, and therefore have achieved little success.

TABLE 1

Composition of the layer diets and layer weight gain

Groups	Diet[1,2]		Body wt		Gain in wt
	Corn	Garlic fractions	Initial[3]	Final[4]	
	%	%	g		%
Corn (control)	78.000	-	459 ± 64[5]	840 ± 95[5]	83
Corn + garlic paste	74.200	3.8	508 ± 84	900 ± 101	77
Corn + garlic PESF	77.980	0.014	487 ± 69	880 ± 97	81
Corn + garlic MESF	77.658	0.341	486 ± 72	853 ± 85	76
Corn + garlic WASF	77.207	0.793	482 ± 59	868 ± 98	80
Corn + garlic residue	77.879	0.121	494 ± 32	892 ± 102	81
Corn + garlic oil (commercial)	77.986	0.014	456 ± 60	824 ± 86	81

TABLE 2

Percent composition of broiler diets, initial and final body weights and feed consumption

Groups	Diet[1,2]		Body weight			
	Corn	Garlic fractions	Initial[3]	Final[4]	Gain in wt	Consumption
		%	g		%	kg
Corn (control)	70.00	-	1414 ± 128	2169 ± 1455	53	18.85
Corn + garlic paste	67.200	3.800	1587 ± 135	2219 ± 115	39[a]	18.60
Corn + garlic PESF	69.986	0.014	1552 ± 74	2296 ± 82	48	18.94
Corn + garlic MESF	69.658	0.342	1640 ± 48	2418—92	47	18.96
Corn + garlic WASF	69.207	0.793	1583 ± 51	2411 ± 99	52	18.95
Corn + garlic residue	69.879	0.121	1516 ± 108	2183 ± 121	44[a]	18.64
Corn + garlic oil (commercial)	69.986	0.014	1598 ± 150	2281 ± 182	43[a]	18.93

1 PESF, MESF and WASF stand for petroleum ether-, methanol- and water-soluble fractions of garlic, respectively. Five grams of garlic bulb yielded 3.89g garlic paste. Fractionation of 3.8g garlic paste yielded 14 mg PESF, 342 mg MESF, 793 mg WASF and 121 g residue. Commercial galric oil was added at the level of the PESF. [2]Each diet also contains soybean meal (44% protein), 23.5%; alfalfa meal (17% protein), 2.0%; meat and bone meal, 2.0%; dicalcium phosphate, 1.0%; calcium carbonate, 0.5%; iodized salt, 0.5%; vitamin and mineral mixture, 0.5%. Vitamin and mineral mixture provides per kilogram diet: vitamin A, 3000 IU; cholecalciferol, 500 IU; riboflavin, 2.5 mg; calcium pantothenate, 3.0 mg; vitamin B_{12} 0.005 mg; zinc sulfate ($ZnSO_4$), 70 mg; and manganese dioxide (MnO_2), 25.0mg, grit (5.0%) was also incorporated at the expense of each diet. [3]Eight weeks of age. [4]Twelve weeks of age. [5]Mean ± SD; n = 8 chickens per layers and 6 broiler group. [a]Significantly different from control at $P < 0.01$.

TABLE 3
Effects of garlic fractions on hepatic anzyme activities in 12-week-old layers[1,2]

Diet	HMG-CoA reductase[3]	Cholesterol 7α-hydroxylase[4]	Fatty acid synthetase[5]	Malic enzyme[6]	Glucose-6-phosphate dehydrogenase[6]	6-Phosphogluconate dehydrogenase[6]
Corn (control)	909 ± 70^{a}(100)[7]	1.14 ± 0.08^{a}(100)	175 ± 14.0^{a}(100)	450.9 ± 42.0^{a}(100)	11.8 ± 1.8^{a}(100)	45.0 ± 7.0^{a}(100)
Corn + garlic paste	253 ± 40^{b}(28)	0.72 ± 0.04^{b}(63)	158 ± 12.0^{ac}(90)	396.6 ± 30.0^{ab}(88)	8.7 ± 0.8^{b}(74)	31.5 ± 3.0^{b}(70)
Corn + garlic PESF	193 ± 30^{c}(21)	0.61 ± 0.03^{c}(54)	124 ± 11.0^{b}(71)	407.6 ± 46.0^{a}(90)	9.1 ± 1.9^{a}(77)	32.4 ± 2.0^{b}(72)
Corn + garlic MESF	159 ± 30^{c}(17)	0.56 ± 0.04^{c}(49)	141 ± 11.0^{c}(81)	331.2 ± 37.0^{b}(73)	7.2 ± 0.7^{b}(61)	33.3 ± 4.0^{b}(74)
Corn + garlic WASF	192 ± 28^{c}(21)	0.65 ± 0.05b^{c}(57)	145 ± 8.0^{c}(83)	368.4 ± 32.0^{b}(82)	7.9 ± 1.8^{b}(67)	30.5 ± 6.0^{bc}(68)
Corn + garlic residue	781 ± 62^{a}(86)	0.93 ± 0.06^{d}(82)	172 ± 13.0^{a}(98)	450.2 ± 45.0^{a}(100)	10.8 ± 2.3^{ab}(92)	38.6 ± 2.0^{ac}(86)
Corn + garlic oil (commercial)	230 ± 30^{b}(26)	0.67 ± 0.05^{c}(59)	160 ± 4.0^{a}(91)	412.4 ± 40.0^{ab}(92)	7.7 ± 1.2^{b}(65)	36.0 ± 4.0^{abc}(80)

[1]Experimental period was 4 weeks; time of killing was 0800. Data expressed as means ± SD; n = 8, HMG-CoA reductase, 3-hydroxy-3-methyglutaryl-CoA reductase. PESF, MESF and WASF stand for petroleum ether, methanol- and water-soluble fractions of garlic, respectively. [2]Values not sharing a common superscript letter are different at $P < 0.01$. [3]Picomoles of mevalonic acid synthesized per minute per milligram of microsomal fraction. [4]Picomoles of [14C] cholesterol into 7α-[14C] hydroxycholesterol per minute per milligram of microsomal fraction. [5]Nanomoles of NADPH oxidized per minute per milligram of cytosolic fraction. [6]Nanomoles of NADPH reduced per minute per milligram of cytosolic fraction. [7]Percentages of respective control activity data are in parentheses.

TABLE 4

Effects of garlic fractions on serum lipids in 12-week-old layers[1,2]

Diet	Concentration in Serum					
	Cholesterol	Triglycerides	HDL-chol	2LDL-chol	Total cholesterol HDL-chol	LDL-chol HDL-chol
Corn (control)	168 ± 2.0^a(100)3	125.1 ± 12.0^a(100)	57.0 ± 4.0^a(100)	86.0 ± 7.0^a(100)	86.0 ± 7.0^a(100)	2.95(100)
Corn + garlic paste	132.8 ± 10.0^b(79)	98.1 ± 10.0^b(78)	55.5 ± 8.0^a(97)	57.7 ± 5.0^b(67)	57.7 ± 5.0^b(67)	2.39(81)
Corn + garlic PESF	128.6 ± 8.0^b(76)	113.1 ± 10.0^a(90)	56.2 ± 8.0^a(99)	59.4 ± 4.0^b(69)	59.4 ± 4.0^b(69)	2.29(78)
Corn + garlic MESF	126.5 ± 4.0^b(75)	92.6 ± 11.08(71)	57.0 ± 5.0^a(100)	51.0 ± 4.0^b(59)	51.0 ± 4.0^b(59)	2.22(75)
Corn + garlic WASF	134.7 ± 11.0^b(80)	96.3 ± 10.0^b(77)	54.7 ± 6.0^a(96)	60.7 ± 5.0^b(71)	60.7 ± 5.0^b(71)	2.46(83)
Corn + garlic residue	152.2 ± 14.0^a(91)	121.7 ± 10.0^a(97)	53.5 ± 4.0^a(94)	74.4 ± 7.0^a(87)	74.4 ± 7.0^a(87)	2.84(96)
Corn + garlic oil (commercial)	128.9 ± 7.0^b(77)	115.1 ± 7.0^a(92)	54.5 ± 3.0^a(96)	61.2 ± 3.0^a(96)	61.2 ± 6.0^b(71)	2.36(80)

[1]Experimental period was 4 weeks; time of killing was 0800 hours. Data are means ± SD; n = 8. PESF, MESF and WASF stand for petroleum ether-, methanol- and water-soluble fractions of garlic, respectively. [2]Values not sharing a common superscript letter are different at $P < 0.01$. [3]Percentages of respective control activity data are in parentheses.

TABLE 5

Effects of garlic fractions on hepatic HMG-CoA reductase activity and serum cholesterol in 12-week-old broilers[1,2]

Diet	HMG-CoA reductase[3]	Serum Cholesterol			
		Total(fasted)	Total (refed)	HDL-chol	LDL-chol
Corn (control)	890 ± 44^{a}(100)[4]	158.3 ± 5^{a}(100)	163.1 ± 6^{a}(100)	59.3 ± 4^{a}(100)	74.6 ± 6^{a}(100)
Corn + garlic paste	448 ± 29^{b}(50)	147.2 ± 4^{b}(93)	149.3 ± 4^{b}(92)	56.2 ± 3^{a}(95)	50.9 ± 7^{b}(68)
Corn + garlic PESF	380 ± 27^{c}(43)	139.7 ± 3^{b}(88)	142.6 ± 3^{b}(87)	56.7 ± 2^{a}(96)	46.8 ± 6^{b}(63)
Corn + garlic MESF	280 ± 27^{d}(32)	122.8 ± 7^{c}(78)	122.2 ± 4^{c}(75)	58.6 ± 3^{a}(99)	42.2 ± 4^{b}(57)
Corn + garlic WASF	300 ± 25^{d}(34)	129.6 ± 2^{c}(82)	132.5 ± 8^{c}(81)	57.3 ± 2^{a}(97)	44.7 ± 5^{b}(60)
Corn + garlic residue	720 ± 40^{e}(81)	152.6 ± 4^{a}(96)	153.7 ± 6^{a}(94)	58.1 ± 5^{a}(98)	66.8 ± 6^{a}(90)
Corn + garlic oil (commercial)	410 ± 25^{b}(46)	137.4 ± 6^{b}(87)	133.8 ± 3^{c}(82)	55.8 ± 4^{a}(94)	57.9 ± 5^{b}(78)

[1]Experimental period was 4 weeks; time of killing was 0800 hours. Data are means ± SD; n = 6. HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; PESF, MESF and WASF stand for petroleum ether-, ethanol- and water-soluble fractions of garlic, respectivley. [2]Values not sharing a common superscript letter are different at $P < 0.01$. [3]Picomoles of mevalonic acid synthesized per minute per milligram of microsomal fractions. [4]Percentages of respective control activity data are in parentheses.

TABLE 6

Effects of garlic fractions on hepatic lipogenic enzymes in 12-week-old broilers[1,2]

Diet	Fatty acid synthetase[3]	Glucose-6-phosphate dehydrogenase[4]	6-Phosphogluconate dehydrogenase[4]	Malic enzyme[4]	Citrate-cleavage enzyme[5]
Corn (control)	246 ± 19.0^a(100)[6]	15.8 ± 0.5^a(100)	48 ± 0.4^a(100)	307.9 ± 26^a(100)	8.6 ± 0.2^a(100)
Corn + garlic paste	182 ± 11.5^b(74)	10.7 ± 0.6^b(68)	3 5/8 $\pm 0.3^b$(68)	268.7 ± 22^a(87)	6.7 ± 0.3^b(78)
Corn + garlic PESF	171 ± 8.0^b(70)	9.2 ± 0.4^b(58)	31 ± 0.7^c(65)	262.3 ± 24^{ab}(85)	6.2 ± 0.4^b(72)
Corn + garlic MESF	118 ± 7.5^c(48)	6.7 ± 0.2^c(42)	24 ± 0.6^d(50)	224.6 ± 17^b(73)	5.0 ± 0.2^c(58)
Corn + garlic WASF	122 ± 9.0^c(50)	7.0 ± 0.3^c(44)	28 ± 1.0^e(58)	263.5 ± 16^{ab}(86)	5.2 ± 0.1^c(60)
Corn + garlic residue	226 ± 18.2^a(92)	13.7 ± 0.6^d(87)	40 ± 1.4^f(83)	267.4 ± 22^a(87)	6.9 ± 0.2^b(80)
Corn + garlic oil (commercial)	167 ± 7.0^b(68)	8.8 ± 0.2^b(56)	30 ± 0.6^c(63)	240.0 ± 24^{ab}(63)	5.9 ± 0.2^b(69)

[1]Experimental period was 4 weeks; time of killing was 0800 hours. Data are means ± SD; $n = 6$. PESF, MESF and WASF stand for petroleum ether-, methanol- and water-soluble fractions of garlic, respectively. [2]Values not sharing a common superscript letter are different at $P < 0.01$. [3]Nanomoles of NADPHoxidized per minute per milligram of cytosolic fraction. [4]Nanomoles of NADPH reduced per minute per milligram of cytosolic fraction. [5]Nanomoles of product formed per minute per milligram of cytosolic fraction. [6]Percentages of respective control activity data are in parentheses.

PHARMACOLOGICAL STUDIES ON EMBLICA OFFICINALIS

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INTRODUCTION

Avicenna (Sheikh Bu Ali Sina, 980-1037 A.D.; Fig. 1) was the author of the 'Canon of Medicine' *(Al-Qanoon)*. In his tract on cardiac drugs he has listed 64 drugs which he used for the treatment of cardiac diseases[1]. A possibility of errors of interpretation exists around the research on such drugs mentioned in old treatise. Research problems pertaining to these drugs can be resolved by applying the modern scientific principles. Attempts have been made to investigate some of the drugs mentioned by Avicenna[2,3,4]. This paper deals with one such drug namely *Emblica officinalis.*

The fruit pulp of *Emblica officinalis* (English: *Embelic myrobalans:* Hindi: *Amla* mentioned hereafter as myrobalans) is one of the important drugs used in the Indian systems of medicine. It is used both as medicine for some diseases and as a tonic to build up lost vitality and vigor[5]. In Unani (Greeco-Arab) system of medicine, it is described as a tonic for heart and brain. Being a rich source of vitamin C it has been successfully used to treat human scurvy[6]. Barring the discovery of ascorbic acid and presence of large amount of tannins, there does not seem to have any detailed work done on the plant. Therefore, the fruit pulp was subjected to chemical and pharmacological investigations.

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* Bulletin of Islamic Medicine, 1: 471 - 478, 1981.

In order to do this, various claims of the practitioners of the indigeneous system of medicine had to be kept in mind in order to devise suitable experiments to their validities. For example, *Chavanaprash*, a preparation containing mainly myrobalans is extensively used in India for chest diseases and for lowered vitality. It was not difficult to devise a test for at least one of these claims, namely, treatment of cough[7]. Screening for anti-bacterial and anti-fungal activity showed mild anti-bacterial activity[8]. The active principle appeared to be present in a fairly concentrated form in a fraction which was prepared by treating 80% alcoholic extract of myrobalans with HCl and extracting with ether. This semi-pure fraction inhibited the growth of *Micrococus pyogens var. aureus, Salmonella typhosa* and *paratyphi* at a concentration of 0.21 mg/ml and that of *M. pyrogens var albus, S. schottmuelleri* and *S. dysenterics* at a concentration of 0.42 mg/ml when tested by agarstreak method.

During the general pharmacological screening of the 80% alcoholic extract of myrobalans for various pharmacodynamic actions, a cardiotonic activity was observed. This property did not appear to be a true cardiotonic activity but was more like the actions of adrenaline[9]. A pure crystalline material was isolated from the alcoholic extract which was a neutral non-nitrogenous substance with significant pharmacological actions. This active principle was designated as 'phyllemblin'.



AVICENNA

AVICENNA: (Arabic: Ibn-e-Sina) was called the "Prince of Physicians". Arab philosopher and physician, considered by some scholars to have been the greatest produced by the culture of the eastern Arab world. He displayed (c 997) his medical proficiency while still a youth, by curing a Persian ruler of a critical illness, and was thereafter variously physician and adviser to rulers at Khiva (c 1004) and Hamadan(until 1037). He was the author of more than 100 works, of which his Canon of Medicine (Qanoon) is unquestionably the most important, and was widely translated in Europe during the Middle Ages.

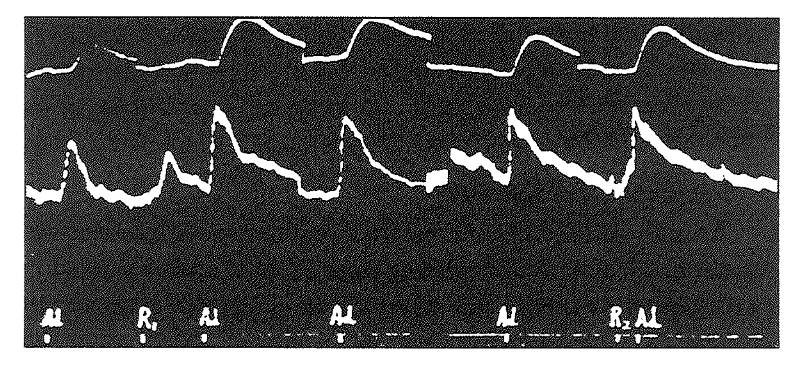


Fig. 2

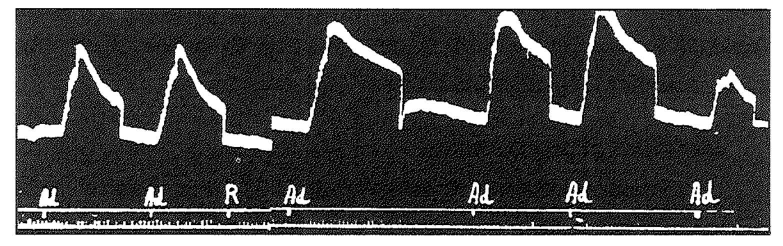


Fig. 3

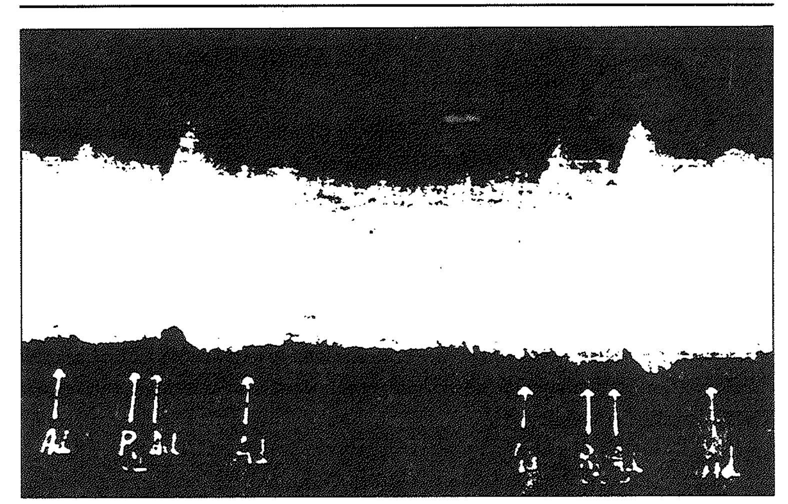


Fig. 4

EXPERIMENTAL

Preparation of Extracts

An authentic sample of dried fruits, cut to remove the seeds was further dried in an air-circulating drier at 40°C and powdered to pass through 64 mesh sieve. The powder (1 kg) was extracted by percolation, successively with petroleum ether (60-80°C), ether and 80% ethyl alcohol. All the extracts were concentrated *in vacuo* and kept in a vacuum dessicator over calcium chloride till the weights were nearly constant. For administration, a quantity of these extracts representing a definite quantity of crude drug was suspended in 1-3 ml distilled water.

Isolation of phyllemblin

Powdered dried pulp of myrobalan (7 kg) was defatted with petroleum ether and percolated with 80% ethyl alcohol. The alcoholic extract was distilled *in vacuo*, the extract suspended in 2.5 litres of 80% ethanol and mixed with a strong solution of potassium

hydroxide (580 ml of 60%) till the pH was 10. It was left for 24 hours in a refrigerator. The mixture was then diluted to 10 litres of distilled water and extracted repeatedly with peroxide-free ether. The combined ether extract was then dried over anhydrous sodium sulphate and distilled. A brownish residue (10g) was obtained. This was washed with cold chloroform which removed the brown coloured matters. The pale residue, insoluble in cold chloroform, was dissolved in boiling chloroform, decolourized with a small quantity of animal charcoal and cooled when phyllemblin crystals appeared as white needles (yield 0.80%).

Pharmacological Testings

Output of respiratory tract fluid: Rabbits of either sex (2-2.5 kg) were anaesthetized with urethane (7 ml of 25% per kg) and the respiratory tract fluid (R.T.F.) was collected according to the method of Boyd and Parry[10].

For *in vivo* experiments cats, dogs and rabbits were used. Cats (3-3.5 kg) were anaesthetized with 85 mg/kg chloralose (intravenously) after initial induction of anaesthesia with ether. Dogs were anaesthetized with 30 mg/kg pentobarbitone sodium intravenously. Rabbits were anaesthetized with 7 ml of 25% urethane intravenously. Studies on blood pressure, respiration, intestinal movement and nictitating membrane were made on cats and dogs according to standard methods. Studies on the rate and depth of respiration was carried out according to the method of Burn[12].

The isolated hind limb of rat and isolated ear of rabbit was perfused with warm oxygenated Ringer's solution and the outflow measured as drops/min or ml/min.

Studies on isolated organs such as the heart of frog and rabbit, auricle of rabbits, duodenum of rabbit, ileum of guinea-pig, seminal vesicle of rat, tracheal chain of guinea-pig and uterus of rat were made according to standard methods.

Acute toxicity of phyllemblin was studied in mice. The drug was administered orally for acute toxicity studies.

RESULTS

Effect on respiratory tract fluid (R.T.F.)

The experimental animals used for expectorant activity differs from the normal animals because it is anaesthetized and is held partially upside down over a period of 6-8 hours which was the period of study in most of these experiments. The mean rate of production of R.T.F. was about 4 ml/kg/24 hrs in rabbits. Each animal served its own control. It was observed that the R.T.F. during the second hour of the experiment could be taken as control value since variation in the output was observed only in the first hour of observation.

Petroleum ether extract, ether extract, 80% alcoholic extract, product of steam distillation of fruit pulp and phyllemblin was tested for expectorant activity.

After taking the R.T.F. reading in the second hour, the drugs were administered orally in a dose equivalent to 4gm of fruit pulp and the effect was noted hourly for next 5 hours. Six rabbits were used for each drug. Results are presented in Table I.

Only 80% alcoholic extract showed an increase in the output of R.T.F. It showed an increase of 60.35% in R.T.F. secretion over the normal. For comparison other known drugs commonly used as expectorant was used. For example, ammonium chloride (400 mg/kg) produced an increase of 36.7% in R.T.F . The results indicated that with the dose given, 80% alcoholic extract of myrobalan produced the highest excretion of the R.T.F .

For the determination of the mode of action, rabbits were given 80% alcoholic extract by different routes viz. (a) orally (b) intraperitoneally (c) intravenously and (d) intraperitoneally after an injection of cholinergic blocking agent like atropine sulphate (2 mg/

kg) or oxyphenonium bromide (2 mg/kg). The dose given intravenously was 25% of that given orally. In all the cases 80% alcoholic extract showed considerable stimulation of R.T.F. excretion and the stimulation was much more when the drug was given parenterally (Table I).

Pharmacodynamic actions of Phyllemblin

Cardiovascular action

Phyllemblin had very little or no effect on the blood pressure of dogs or cats (Fig. 2). However, it potentiated the pressure response of 1 ug of adrenaline on the blood pressure of cat. Dose of 5 mg phyllemblin produced significant potentiation of the action of adrenaline in normal anaesthetized cats and in spinal cat preparation (Fig. 3).

Small doses of phyllemblin (2 and 3 mg) showed a negligible stimulation of isolated perfused frog heart while 4 and 5mg showed an inhibition. Phyllemblin showed a significant potentiation of the effect of 0.1 ug adrenaline at doses of 100 and 500 ug (Fig. 4). On isolated perfused rabbit heart, phyllemblin produced stimulation of the heart movement at the doses of 50, 100, 500 and 1000 ug (Fig. 5). Phyllemblin (10 and 20 mg) potentiated the stimulant effect of 20 ug adrenaline on the isolated auricle of rabbit.

In isolated frog heart perfused with normal Ringer's solution phyllemblin (1 mg) increased the cardiac outflow from a normal of 5 ml/min to 6.5 ml/min. Comparable increase in the outlfow was observed with 1 ug of adrenaline. In another set of experiment where frog heart was perfused *in situ* with normal Ringer's solution, the outflow was 13 ml/min. When the normal Ringer was replaced with 1/4th calcium Ringer, the cardiac outflow decreased from 13ml to 2 ml/min. When 1 mg/ml of phyllemblin was administered at this stage, the cardiac outflow increased from 2ml to 9 ml/min.

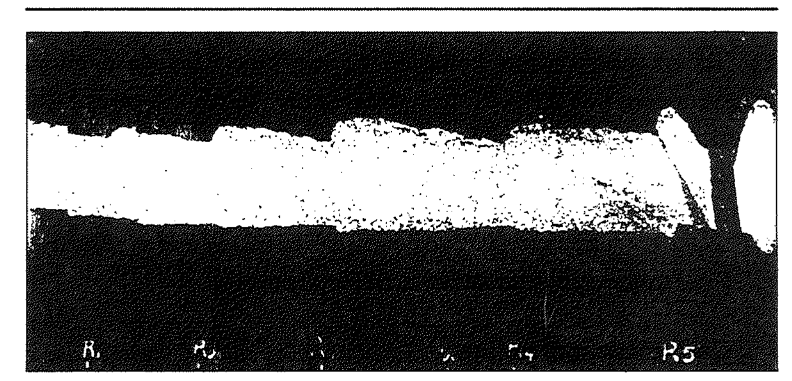


Fig. 5

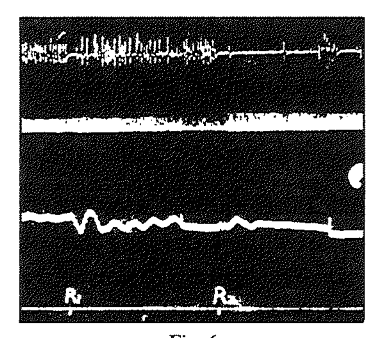


Fig. 6

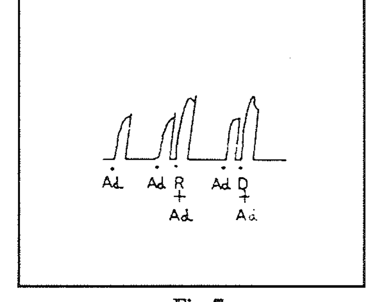


Fig. 7

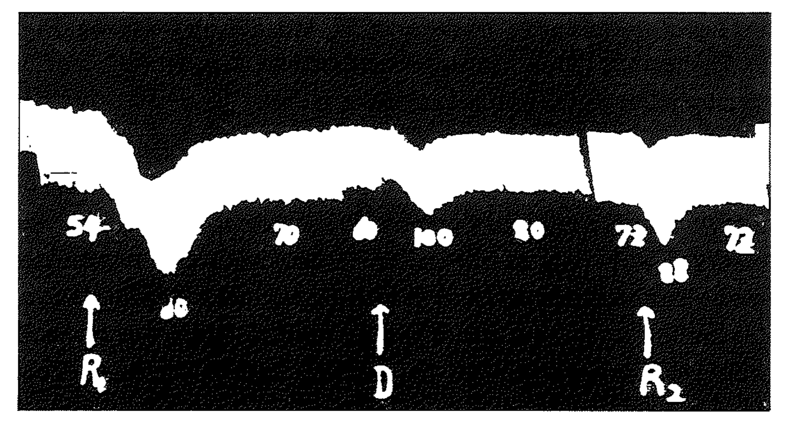


Fig. 8

Phyllemblin (5 mg) decreased the outflow of perfused rat hind limb. The decrease was from a normal of 285 drops/5 min to 180 drops/5 min. Under similar condition 2 ug adrenaline decreased the outflow to 82 drops/min. Similarly, on perfused isolated ear of rabbit, phyllemblin (3 mg) decreased the outflow from a normal of 170 drops/6 min to 64 drops/6 min whereas adrenaline (2 ug) reduced the outflow to 38 drops/6 min.

Intestinal smooth muscle:

Phyllemblin (28 mg) arrested the intestinal movement *in vivo* in cat (Fig. 6). In doses of 1, 2, 3, and 4 mg/25 ml bath it produced relaxation of isolated duodenum of rabbit and markedly potentiated the relaxant effect of 0.5 ug of adrenaline in doses of 50, 100 and 200 ug. Phyllemblin inhibited the spasm induced by actylcholine, histamine and barium chloride on guinea-pig ileum. In doses of 1.8, 0.55 and 0.30 mg/25 ml bath fluid, phyllemblin produced 50% blocking of the action of a standard dose of histamine acid phosphate (10 ug), actylcholine (10 ug) and barium chloride (100 ug) respectively.

Nictitating membrane of cat:

Phyllemblin (10 mg) itself produced mild contraction of the nictitating membrane and also potentiated the effect of adrenaline (5 ug) at doses of 10 and 15 mg (Fig. 2).

Seminal vesicle of rat:

Phyllemblin *per se* did not produce contraction of isolated seminal vesicle of rat but significantly potentiated the effect of 10 ug adrenaline. The adrenergic potentiating activity of 500 ug of phyllemblin was comparable with 100 ug of ephedrine hydrochloride (Fig. 7). Ascorbic acid (40 mg), gallic acid (40 mg) and tannic acid (40 mg) present in myrobalan did not potentiate the action of 10 ug adrenaline.

Isolated tracheal chain of guinea-pig, and rabbit respiration

Phyllemblin (4 mg/25 ml bath) completely blocked the action of 20 ug histamine acid phosphate on isolated tracheal chain. Ephedrine HCl (2 mg) produced inhibition of the histamine equivalent to that produced by 2 mg phyllemblin.

Phyllemblin (50 mg) produced marked relaxation of the respiratory movement in anaesthetized rabbits and the respiratory rate increased from 54 to 80 per minute. Ephedrine (5 mg) caused relaxation of respiratory movement and respiratory rate increased from 60 to 100/min. A comparable dose of phyllemblin (5 mg) produced equal or a slightly more relaxation (Fig. 8).

Acute Toxicity

Phyllemblin was well tolerated by mice in doses up to 100 mg/kg when given intraperitoneally and up to 500 mg/kg when given orally. At doses above 500 mg/kg animals appeared drowsy and dull soon after the injection and were active again after 1-3 hours.

DISCUSSION

Expectorant activity of the 80% alcoholic extract of the fruit pulp of *Emblica officinalis* was studied by the technique proposed by Boyd and Perry[10] which is fairly simple and reproducible. Of the several fractions studied, only 80% alcoholic extract showed activity. Phyllemblin was not active. Gallic acid, tannins and ascorbic acid present in the extract were also tested and found inactive. An attempt was made to study the mode of action. The alcoholic extract was effective orally, intraperitoneally, intravenously and even in atropinized animals. This is unlike ammonium chloride, which is known to act as a reflex stimulating agent of bronchial secretion through the irritation of the gastric mucosa and is inactive when given parenterally. Since the action of the extract was not blocked by cholinergic blocking agents, it was inferrered

that like eucalyptol (Boyd and Pearson[13]) it directly stimulated the bronchial glands.

The pharmacodynamic actions of phyllemblin (a white crystalline compound, m.p. 161-3°C isolated from 80% alcoholic extract) can be grouped into two classes (i) direct action on various systems, (ii) potentiation of the actions of adrenaline. Of the direct effect, mention may be made of the mild stimulation of isolated heart of frog and rabbit, short rise in cat's blood pressure, contraction of the nictitating membrane, reduction in the outflow of the perfused isolated hind limb of rat and ear of rabbit, increase in cardiac outflow of frog heart and antispasmodic action on intestinal smooth muscle. The indirect action include potentiation of the action of adrenaline on the blood pressure of cat, isolated frog heart, nictitating membrane of cat, rabbit intestine and seminal vesicles of rat.

Phyllemblin resembles adrenaline in its direct effects, but it does not resemble in other details. For example, *per se* it does not elevate the blood pressure, does not contract the seminal vesicle and does not elevate the blood glucose level. It resembles ephedrine in its ability to potentiate adrenaline. But differs from ephedrine in some respects; for example, it does not show techyphylaxis. It stimulates the heart, produces coronary dilation and peripheral vasoconstriction. Therefore, its action is adrenergic but it is neither completely like adrenaline nor like ephedrine.

The antispasmodic activity and adrenergic potentiating activity is comparable in many ways to rutin and other flavanoid compounds. However, it was found that phyllemblin had no effect on capillary permeability. It has been argued that rutin exerts its adrenergic potentiating activity mainly due to its anti-oxidant property.

CONCLUSIONS

Fruit pulp of *Emblica officinalis* is a rich source of vitamin C and provides vitamin C in most stable form. Its bio-availability in cases of pulmonary tuberculosis is much better than synthetic vitamin C. It possesses powerful expectorant activity by directly stimulating the mucous cell of the bronchial tree. In addition, the extract has mild antibacterial activity.

Phyllemblin, an active principle isolated from the 80% alcoholic extract of *Emblica officinalis* acts on cardiovascular and other systems partly like adrenaline and partly like ephedrine. The investigations support the use of this drug by Avicenna (Sheikh Bu Ali Sina) in the treatment of cardiovascular diseases and its present use in cardiovascular and chest diseases in the Indian systems of medicine.

ACKNOWLEDGEMENTS

I wish to thank Hakeem Abdul Hameed, President, Institute of History of Medicine & Medical Research, New Delhi, India for his suggestion to undertake the scientific evaluation of the drugs used by Sheikh Bu Ali Sina (Avicenna) by using modern techniques. It was his dedication and keen interest for the work of Avicenna which inspired me to take up the investigations on cardiac drugs mentioned by Avicenna.

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ANTISECRETORY PROPERTIES OF ACHYRANTHES ASPERA

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ANTISECRETORY PROPERTIES OF ACHYRANTHES ASPERA*

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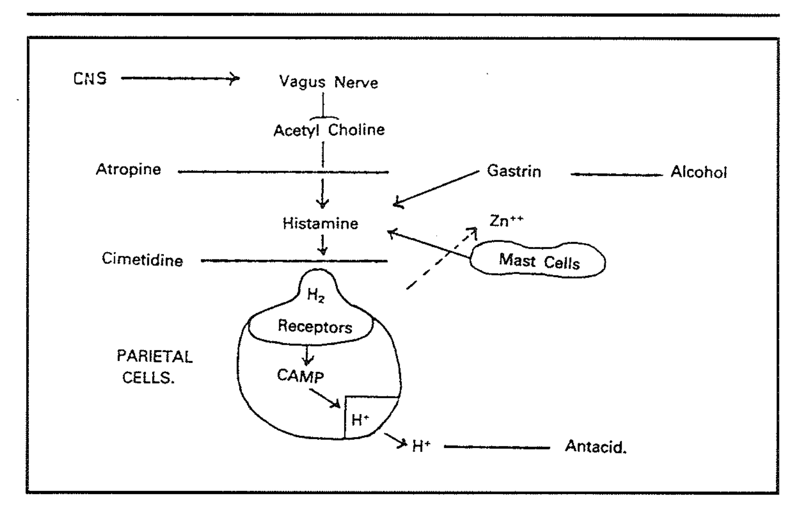
The plant *Achyrenthes aspera* (F. Amaranthaceae) is an important medicinal plant used in the indigenous systems of medicine, especially in the Islamic and Unani System of Medicine. The plant is known as *Atkumah* in Arabic, Prickly Chaff-flower in English and *Chirchita* in Hindi and in Urdu.

The whole plant and its parts - leaves, seeds and roots - have been in use in various indigenous systems of medicines in India for treating a large number of diseases, including leprosy, bowel complaints, general anasarca and arthritis[1,2,3]. The plant has been claimed by Wahid and Siddiqui[4] as an antacid and this property has been attributed to it in Unani System of Medicine. *Chirchita* is being used in many Ayurvedic and Unani formulations, such as Cystone, Kushta Sam-ul-Far etc., which are popularly prescribed even by allopathic doctors to cure different ailments.

Since the biological findings supporting this claim were not available, the present work was undertaken to investigate the usefulness of the drug in hyperacidity syndrome.

Acid secretion is a complex process which is mediated through a number of mediators. A schematic representation of mode of acid secretion is given below:

* Bulletin of Islamic Medicine, 3: 444-454, 1984.



MATERIAL AND METHODS

The plants with fully developed spikes were collected from area around Hamdard College of Pharmacy, Hamdard Nagar, New Delhi, during the months of October - December. The collected plants were brushed off the dust and cut into small pieces after removing the insect-eaten leaves by hand. The cutpieces of plant were dried at 60°C in a hot air oven. The dried plant material was powdered in Hammer mill and then sifted through sieve 40.

The plant constituents studied for antisecretory properties were:-

A - *Crude Aqueous Extract*: It was prepared by boiling 100g of the powdered material with distilled water for three hours. The water extract was filtered and the filtrate was evaporated to dryness on a water bath when a dark brownish-black residue (9.5g) was obtained. The dried extract gave positive tests for alkaloids, saponins and reducing sugars. The residue was tested for its effects on gastric acidity at 250 and 500mg/kg dose levels given orally to the rabbits. The extract was dissolved in water and then given to rabbits through Ryle's tubes.

B - *Chloroform Solube Alkaloids:* They were extracted by a method similar to that reported by Basu[5]. The dried plant material

(1.5kg) was extracted with chloroform. The chloroform layer was concentrated and poured into 200ml 2% v/v sulphuric acid and shaken for 2 hours mechanically. The acid layer was collected by filtration. The residue was again extracted with 2% sulphuric acid successively until all the alkaloids were extracted (till Dragondorff's test was negative). The acid layer was extracted with chloroform repeatedly to remove chloroform soluble non-alkaloidal matter. Then the acid layer was made alkaline by adding sodium carbonate and the resultant alkaline layer was extracted with 50ml chloroform repeatedly till a negative test with Dragondorff's reagent was obtained. Chloroform layers were collected, dried over anhydrous sodium sulphate and evaporated to dryness over a water bath when a yellowish-brown residue was obtained. This residue was dissolved in 50ml 2% sulphuric acid and the acid layer extracted with chloroform to remove impurities. Then it was made alkaline and extracted with chloroform to exhaustion. The chloroform layers were combined and dried over anhydrous sodium sulphate. The chloroform was recovered, when a pale yellow resinous mass was obtained. This mass was dissolved in a little ethanol to which excess of solvent ether was added. A pure alkaloidal precipitate (0.185/g) was obtained, which was tested for pharmacological effect.

PHARMACOLOGICAL STUDIES

The method adopted for studying the effect of drugs on gastric acid secretion was similar to that reported by Curwaine and Turner[6].

Male rabbits weighing between 1 and 2 kg (usually about 1.3 kg) were taken. They were deprived of meals for 18 hours prior to anaesthetizing. The fasted rabbits were anaesthetised with urethane (1.5 g/kg). The anaesthetised rabbits were laid on their back on Brody's table. The body temperature was maintained at 37°C ± 1 °C. A midline abdominal incision was given. Through this incision, the duodenum was gently picked up and the pyloric end of stomach was looped around with a cotton thread taking care not to cause any injury to the adjacent mesentric vessels. Finally the thread was

tied to its opposite ends. The duodenum was then placed into its original position and the abdominal incision was sutured.

The residual contents of stomach were removed using a Ryle's tube and the stomach was washed with normal saline. A test meal (50ml 5% v/v alcohol) was given through the Ryle's tube and after every 15 minutes samples were withdrawn and analysed for free and total gastric acidity by the method described by Tikekar[7].

RESULTS AND DISCUSSION

All the data obtained was subjected to statistical analysis applying Student's 't' test. The results are given in Tables 1 to 14.

The study of the effect of crude aqueous extract, chloroform soluble alkaloids and cimetidine on gastric acid secretion in rabbits induced by 5% ethanol indicated that all the samples and standard drugs tested have an inhibitory effect on acid secretion.

It was observed that crude aqueous extract at 250mg/kg oral dose showed a better and statistically significant reduction in free and total gastric acidity compared to control group, whereas at 500mg/kg oral dose the crude aqueous extract had inhibitory effect on gastric acid secretion but the effect was not statistically significant.

The chloroform soluble alkaloid at 10mg/kg dose when given intraperitoneally to rabbits caused a reduction in gastric acidity but the reduction was not statistically significant.

Chloroform soluble alkaloids had been earlier reported by Kapoor and Singh[8] to posses anti-cholinergic properties, which might have contributed to the anti-secretory property. However, further study is needed to confirm the mode of action of chloroform soluble alkaloids.

The inhibitory effect of crude aqueous extract was compared with cimetidine - a well-known potent H_2-receptor antagonist. It was observed that at 0.5uM/kg dose the effect of cimetidine was comparable with 250mg/kg oral dose of crude aqueous extract.

Zinc element has the property of preventing the acid secretion induced by a variety of agents[9,10]. Since no report on zinc content in the plant was available, the whole plant ash was analysed by Atomic Absorption Spectroscopy for zinc content. It was found to be 0.018% w/w and could in addition be responsible for the anti-secretory property of the drug.

Preliminary studies have also shown that the aqueous extract of the plant inhibited the acetyl choline induced spasm of the rat uterus muscle.

From the above investigation, it is clear that *Chirchit (Achyranthes aspera)* has been rightly classified and used as an anti-secretory drug in the Islamic System of Medicine[4].

SUMMARY

1. The chloroform soluble alkaloid isolated from whole plant as well as from the leaves was found to interfere with the release of gastric hydrochloric acid at 10mg/kg dose level.
2. The crude aqueous extract of whole plant was tested for chemical constituents and for antisecretory properties. The extract was found to contain alkaloids, saponins, reducing sugars and colouring matters.
3. The extract reduced the alcohol induced gastric acidity and the results were statistically significant.
4. Zinc was quantitatively detected in the plant ash.
5. The presence of Zn^{++} element in the plant could also be contributing to the antisecretory property.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to Hakim Abdul Hameed, Chairman, Hamdard College of Pharmacy, for suggesting the problem and extending help and co-operation during the course of investigation.

TABLE 1
CONTROL GROUP
FREE GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S.No. of Individual Rabbit						Mean ± S.E.M
	1	2	3	4	5	6	
15	4.27	4.72	9.38	11.90	4.50	4.60	6.56 ± 1.33
30	7.32	7.08	10.05	13.60	6.00	5.46	8.25 ± 1.25
45	10.98	8.26	9.35	16.15	6.00	5.46	9.37 ± 1.60
60	12.81	8.85	12.06	16.15	6.00	5.46	10.22 ± 1.71
75	14.03	10.03	12.06	17.85	6.00	6.24	11.04 ± 1.88
90	12.20	10.62	9.35	20.40	5.25	7.80	10.94 ± 2.12
105	14.03	11.21	11.39	16.15	5.25	7.80	10.97 ± 1.62
120	12.81	11.21	12.73	16.15	5.25	10.92	11.51 ± 1.42

TABLE 2
CONTROL GROUP
TOTAL GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time Minutes	S.No. of Individual Rabbit						Mean ± S.E.M.
	1	2	3	4	5	6	
15	6.10	5.90	10.72	14.45	5.25	6.24	8.11 ± 1.50
30	8.54	7.67	12.73	16.15	6.75	7.80	9.94 ± 1.51
45	12.81	9.44	12.73	18.70	7.50	7.02	11.37 ± 1.78
60	14.64	10.03	14.07	18.70	7.50	7.02	11.99 ± 1.87
75	15.25	11.21	14.74	19.55	7.50	7.02	12.55 ± 1.99
90	16.47	11.80	12.73	22.95	7.50	9.36	13.47 ± 2.27
105	15.86	12.39	13.40	18.70	6.75	10.14	12.87 ± 1.72
120	17.08	12.39	14.07	18.70	6.75	14.82	13.97 ± 1.71

TABLE 3
CRUDE AQUEOUS EXTRACT 250 mg/kg ORALLY
FREE GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit				Mean ± S.E.M.	P.Value
	1	2	3	4		
15	2.06	0.93	1.98	1.86	1.70 ± 0.26	P < 0.001
30	4.12	1.86	3.96	3.72	3.41 ± 0.55	P < 0.001
60	6.18	3.72	3.95	4.64	4.62 ± 0.55	P < 0.025
90	9.27	4.64	3.96	5.58	5.86 ± 1.17	N.S.
120	10.30	4.64	4.95	6.50	6.60 ± 1.30	P < 0.05

N.S. NOT SIGNIFICANT.

TABLE 4
CRUDE AQUEOUS EXTRACT 250 mg/kg ORALLY
TOTAL GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S.No. of Individual Rabbit				Mean ± S.E.M.	P. Value
	1	2	3	4		
15	3.09	1.86	2.97	2.79	2.67 ± 0.28	P < 0.001
30	6.18	3.72	4.94	4.64	4.87 ± 0.51	P < 0.025
60	8.24	5.58	5.94	6.51	6.56 ± 0.59	P < 0.025
90	11.33	7.44	5.94	6.50	7.80 ± 1.21	N.S.
120	12.35	6.50	6.92	8.37	8.53 ± 1.33	P < 0.05

N.S. NOT SIGNIFICANT.

TABLE 5

CRUDE AQUEOUS EXTRACT 500 mg/kg ORALLY

FREE GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S.No. of Individual Rabbit				Mean ± S.E.M.	P. Value
	1	2	3	4		
15	3.56	2.43	6.32	3.44	3.94 ± 0.83	N.S.
30	3.56	2.43	6.32	5.16	4.37 ± 0.86	P < 0.05
60	3.56	4.05	7.90	7.74	5.81 ± 1.16	N.S.
90	5.34	4.05	7.90	8.60	6.47 ± 1.06	N.S.
120	5.34	4.05	8.69	7.74	6.45 ± 1.06	P < 0.025

N.S. NOT SIGNIFICANT.

TABLE 6

CRUDE AQUEOUS EXTRACT 500 mg/kg ORALLY

TOTAL GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit				Mean ± S.E.M.	P. Value
	1	2	3	4		
15	4.45	4.05	7.90	5.16	5.39 ± 1.73	N.S.
30	5.34	4.45	7.90	6.88	6.14 ± 1.54	N.S.
60	5.34	5.67	9.48	9.46	7.49 ± 1.15	N.S.
90	6.23	5.67	10.27	10.32	8.12 ± 1.25	N.S.
120	6.23	5.69	11.06	11.18	8.53 ± 1.50	P < 0.05

N.S. NOT SIGNIFICANT.

TABLE 7

CHLOROFORM SOLUBLE ALKALOID 10 mg /kg I.P.

FREE GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit					Mean ± S.E.M.	P. Value
	1	2	3	4	5		
15	1.77	6.49	6.70	6.56	2.80	4.86 ± 1.05	N.S.
30	3.54	9.44	9.38	9.84	4.20	7.28 ± 1.56	N.S.
45	5.31	10.25	10.05	10.66	4.20	8.28 ± 1.46	N.S.
60	6.49	12.88	8.71	11.48	4.20	8.75 ± 1.58	N.S.
75	7.67	14.75	9.38	12.30	4.20	9.66 ± 1.83	N.S.
90	7.67	12.98	9.38	11.48	4.20	9.14 ± 1.53	N.S.
105	6.49	14.16	7.37	12.30	4.20	9.98 ± 1.86	N.S.
120	5.31	13.57	5.36	11.48	4.20	7.98 ± 1.89	N.S.

N.S. NOT SIGNIFICANT

TABLE 8

CHLOROFORM SOLUBLE ALKALOID 10mg/kg I.P.

TOTAL GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S.No. of Individual Rabbit					Mean ± S.E.M.	P. Value
	1	2	3	4	5		
15	2.95	7.67	9.72	7.38	3.50	6.24 ± 1.30	N.S.
30	4.72	10.62	12.73	11.48	5.60	9.03 ± 1.62	N.S.
45	6.49	12.39	12.73	11.48	4.90	9.60 ± 1.63	N.S.
60	7.67	14.16	11.34	13.12	4.90	10.24 ± 1.73	N.S.
75	8.85	15.93	10.72	13.12	4.90	10.70 ± 1.87	N.S.
90	8.85	14.10	11.34	13.12	5.60	10.60 ± 1.54	N.S.
105	7.67	15.93	9.38	13.12	4.90	10.20 ± 1.96	N.S.
120	6.49	14.75	8.04	13.12	4.90	9.46 ± 1.91	N.S.

N.S. NOT SIGNIFICANT.

TABLE 9

CIMETIDINE 0.5 uM/kg I.V.

FREE GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit				Mean ± S.E.M.	P. Value
	1	2	3	4 15		
30	2.43	3.36	2.67	4.08	3.13 ± 0.37	P < 0.05
45	4.05	3.36	3.56	5.10	4.02 ± 0.39	P < 0.025
60	3.24	4.20	4.45	5.10	4.25 ± 0.38	P < 0.025
75	8.10	4.20	5.34	5.10	5.68 ± 0.84	P < 0.05
90	8.10	4.20	6.23	5.10	5.91 ± 0.84	P < 0.025
105	6.48	4.20	6.23	4.08	5.25 ± 0.64	P < 0.05
120	5.67	5.04	9.79	5.10	6.40 ± 1.74	N.S.
	7.29	5.88	8.01	5.10	5.57 ± 0.66	P < 0.025

N.S. NOT SIGNIFICANT.

TABLE 10

CIMETIDINE 0.5 uM/kg I.V.

TOTAL GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit				Mean ± S.E.M.	P. Value
	1	2	3	4		
15	4.05	5.04	3.92	6.12	4.78 ± 0.51	N.S.
30	5.18	5.88	5.34	6.12	5.63 ± 0.22	P < 0.025
45	5.18	5.88	5.34	6.12	5.63 ± 0.22	P < 0.025
60	11.34	5.88	7.12	6.53	7.72 ± 1.23	N.S.
75	9.72	6.72	8.01	6.12	7.64 ± 0.80	N.S.
90	8.91	6.72	8.90	6.12	7.66 ± 0.73	P < 0.05
105	7.29	7.56	11.57	7.14	8.39 ± 1.06	N.S.
120	10.53	7.56	10.68	7.14	8.98 ± 0.94	P < 0.025

N.S. NOT SIGNIFICANT.

TABLE 11

CIMETIDINE 1.0 uM/kg I.V.

FREE GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit				Mean ± S.E.M.	P. Value
	1	2	3	4		
15	6.64	2.61	4.00	2.25	3.88 ± 0.95	N.S.
30	6.64	5.22	5.60	3.75	5.30 ± 0.60	N.S.
45	6.64	4.75	5.60	3.00	4.90 ± 0.77	P < 0.05
60	6.64	4.35	6.40	3.00	5.10 ± 0.87	P < 0.05
75	6.64	4.35	7.20	3.00	5.29 ± 0.98	P < 0.05
90	6.64	3.48	6.40	3.00	4.88 ± 0.95	P < 0.05
105	6.64	3.48	8.00	3.00	5.28 ± 1.21	P < 0.025
120	4.15	3.48	7.20	3.00	4.45 ± 0.94	P < 0.005

N.S. NOT SIGNIFICANT.

TABLE 12

CIMETIDINE 1.0 uM/kg I.V.

TOTAL GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit				Means ± S.E.M.	P. Value
	1	2	3	4		
15	7.47	4.35	6.40	4.50	5.68 ± 0.76	N.S.
30	7.47	7.83	8.00	6.00	7.32 ± 0.45	N.S.
45	8.30	6.09	8.00	6.00	7.10 ± 0.61	N.S.
60	7.47	6.09	9.60	5.25	7.10 ± 0.95	P < 0.05
75	8.30	5.22	8.80	5.25	6.89 ± 0.96	P < 0.05
90	8.30	5.22	8.80	6.00	7.08 ± 0.87	P < 0.05
105	7.47	5.22	10.40	5.25	7.08 ± 1.22	P < 0.025
120	5.81	6.09	10.40	6.00	7.07 ± 1.11	P < 0.001

N.S. NOT SIGNIFICANT.

TABLE 13

CIMETIDINE 1.4 uM/kg I.V.

FREE GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit				Means ± S.E.M.	P. Value
	1	2	3	4		
15	5.88	5.10	6.80	4.40	5.54 ± 0.51	N.S.
30	5.88	5.95	6.80	6.16	6.20 ± 0.21	N.S.
45	8.40	7.65	6.80	7.04	7.47 ± 0.36	N.S.
60	10.08	8.50	13.60	6.16	9.58 ± 1.56	N.S.
75	9.24	8.50	12.75	5.28	9.94 ± 1.53	N.S.
90	11.76	9.35	11.90	5.28	9.57 ± 1.55	N.S.
105	10.08	9.35	9.35	4.40	8.30 ± 1.31	N.S.
120	10.08	10.20	7.65	4.40	8.08 ± 1.36	N.S.

N.S. NOT SIGNIFICANT.

TABLE 14

CIMETIDINE 1.4 uM/kg I.V.

TOTAL GASTRIC ACIDITY IN MILLI EQUIVALENTS OF HCl/LITRE

Time in Minutes	S. No. of Individual Rabbit				Mean ± S.E.M.	P. Value
	1	2	3	4		
15	7.06	6.37	8.50	5.28	6.80 ± 0.67	N.S.
30	7.90	7.65	8.50	7.92	7.99 ± 0.18	N.S.
45	10.08	9.35	8.50	7.92	8.96 ± 0.47	N.S.
60	11.25	10.20	17.85	7.92	11.80 ± 2.13	N.S.
75	10.92	16.20	16.15	7.04	11.07 ± 1.89	N.S.
90	12.60	11.05	13.60	7.04	11.07 ± 1.44	N.S.
105	11.76	11.05	13.60	6.16	10.64 ± 1.59	N.S.
120	11.16	11.90	10.20	5.28	9.78 ± 1.49	N.S.

N.S. NOT SIGNIFICANT.

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SIWAK - AS AN ORAL HEALTH DEVICE (PRELIMINARY CHEMICAL AND CLINICAL EVALUATION)

Drs. M. Ragaii El-Mostehy, A.A.Al-Jassem, I.A.Al-Yassin, A.R. EL-Gindy and E. Shoukry

KUWAIT

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A variety of oral hygiene measures have been performed since the dawn of civilization. This has been verified by various excavations done all over the world, in which toothpicks, chewsticks, tree twigs, linen strips, bird's feathers, animal bones and porcupine quills were recovered[1].

Those that originated from plants are tasty twigs and although primitive they represented a transitional step towards the modern toothbrush. It has been stated that about seventeen plants could be enumerated as natural sources for several of these oral hygiene devices [2].

The most widely used tree twigs since early times is the *"Siwak"* or *"Miswak"*[3]. The stick is obtained from a plant called *Salvadora persica* that grows around Mecca and the Middle East area in general[4]. It is widely used among Moslems after Prophet Mohammed (ﷺ) realised its value as a device which should be used by Moslems to clean their teeth. In this respect our Prophet (ﷺ) is considered the first dental educator in proper oral hygiene.

Although there is no reference to the use of *Siwak* in Al-Quran, yet several quotations could be read in the compendium of the sayings of Prophet Mohammed (ﷺ) as to the benefits of *Siwak* in cleanliness[5].

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* Bulletin of Islamic Medicine, 1: 344 - 352, 1981.

One saying reads as follows:

> *"if it were not too much a burden on the believers, I would prescribe that they use the siwak before each prayer".*

Several anecdotes[6], incidents, poems[7], and rules of ethics in using *Siwak* were mentioned in various references talking on the subject of cleanliness of the mouth.

Salvadora persica is in fact a small tree or shrub with a crooked trunk, seldom more than one foot in diameter, bark scabrous and cracked, whitish with pendulous extrimities. The root bark is light brown and the inner surfaces are white, odour like cress and taste is warm and pungent. Chemically the air dried stem bark of *S. persica* is extracted with 80% alcohol and then extracted with ether and run through exhaustive chemical procedures. This showed that it is composed of:

1 - Trimethylamine.

2 - An alkaloid which may be salvadorine.

3 - Chlorides.

4 - High amounts of fluoride and silica.

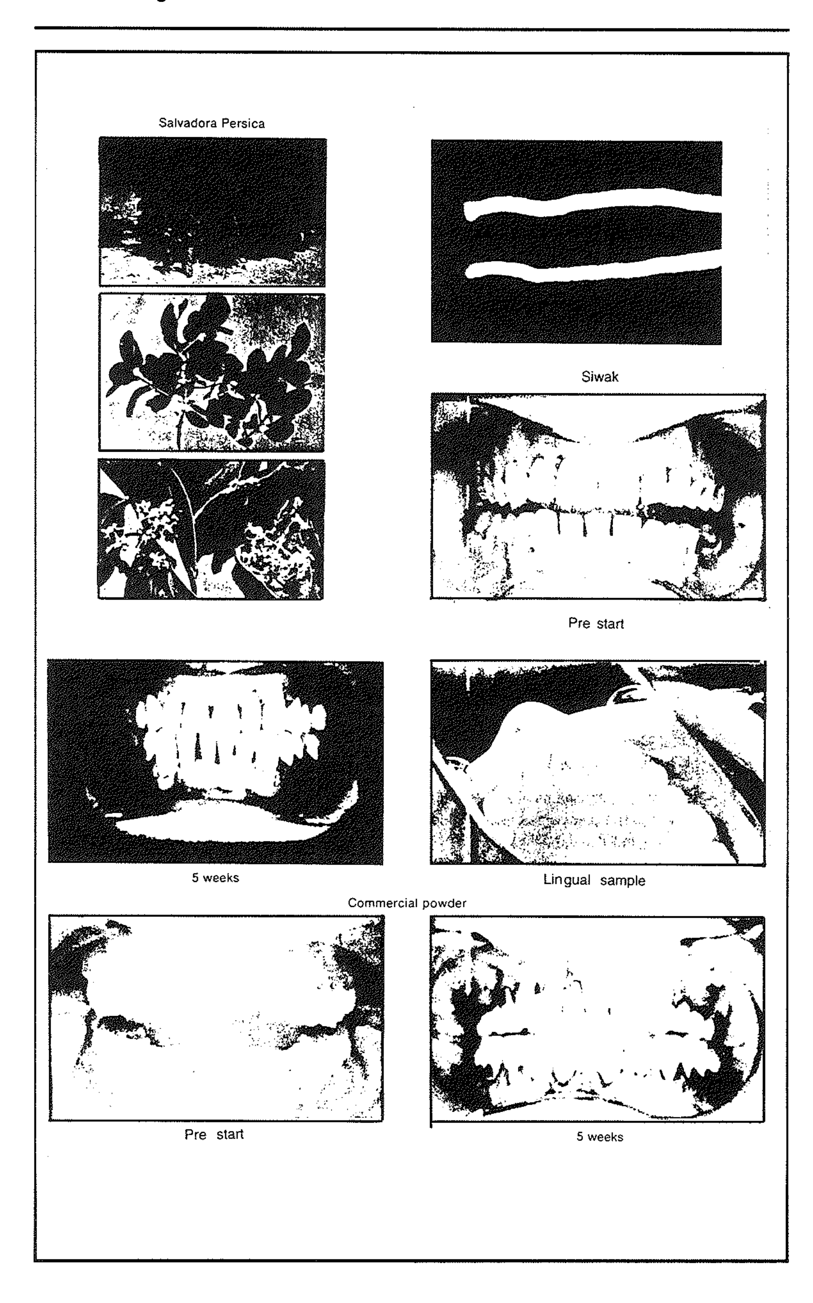
5 - Sulphur.

6 - Vitamin C.

7 - Small amounts of tannins, saponins, flavanoids and sterols.

PURPOSE OF THE PRESENT INVESTIGATION

Because of the great quality of oral cleanliness noticed in individuals who use *Siwak* as the sole device to brush their teeth and because of the low incidence of dental decay of these individuals this work was undertaken.


Salvadora Persica
Siwak
Pre start
5 weeks
Lingual sample
Commercial powder
Pre start
5 weeks

It was intended to study the following:

1 - The mechanical ability of *Siwak* as a cleaning device to the mouth and its ability to rid the mouth of bacterial plaque (aggregates harmful to the gum).
2 - If *Siwak* is powdered and used with a tooth brush, could it act as an efficient mouth cleaner?
3 - As compared to other strongly abrasive toothpowders, could *Siwak* rank as highly efficient as to the used material?

SUBJECTS, MATERIALS AND METHODS

Participants in this trial were 80 individuals (53 male and and 27 females) attending the Periodontology Unit in the Dental Center, Kuwait for regular periodontol checkups. Their age varied from 25 to 55 years and were healthy as proven by their past and present medical histories.

At the first visit all participants received initial preparation of their mouths in the form of thorough scaling and polishing. The plaque percentage index* of each patient scored 'Zero' after several check up visits (2-4 visits to some individuals).

They were divided into 4 groups (20 each).

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* (using a disclosing tablet which has an affinity to stain the the dental plaque)

0 = no plaque

1 = separate flocks of plaque at the necks of teeth.

2 = thin continuous band of plaque (upto 1 mm) at the necks of teeth.

3 = band of plaque wider than 1 mm but covering less than 1/3 of tooth crown.

4 = plaque covering at least 1/3 but less than 2/3 of crown of teeth.

5 = plaque covering 2/3or more of the crown of tooth.

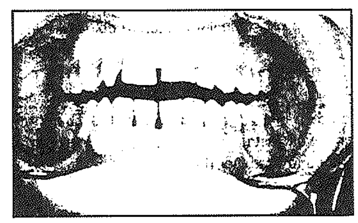
Plaque percentage score was obtained by dividing the total score by the no. of examined tooth surfaces and multiplied by 100.

Starch



Pre start

5 weeks

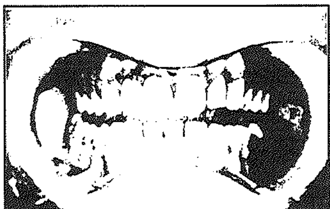


another case of a starch user.

Powdered siwak



Pre start



5 weeks



Lingual sample

I The *Siwak* Group

Individuals were instructed to use *Siwak* as it is and in their own way as the majority were *Siwak* users (15 individuals)

II The powdered *Siwak* group

Siwak was powdered, sieved and packed in 50 gm boxes. Each individual received the powdered *Siwak* and a soft toothbrush and was instructed to use the intrasulcular technique as demonstrated to the individual by one examiner (M.R.E.). Participants ware reviewed prior to the experimental 'Zero' date to detect the inaccuracy of the taught brushing technique.

III Starch group

Each individual in this group was given 50 gm of starch in a container and a soft tootgbrush and was instructed to use the intrasulcular technique of toothbrushing as demonstrated by one examiner (M.R.E.).

IV Commercial tooth powder group

Each individual in this group was given 50 gm of a commercial toothpowder and a soft toothbrush and was instructed to use the intrasulcular technique of toothbrushing as demonstrated by one examiner (M.R.E.).

All participants were exemined every week and the following parameters were recorded and entered in special charts:

1 - Plaque percentage score (discussed before)
2 - Gingival percentage score*
3 - A clinical photograph for individual at each visit after using a disclosing tablet that makes the adherent plaque on teeth visible (red-cote).

FINDINGS

All participants were considered having a 'Zero' plaque percentage score as a baseline. As regards the gingival percentage score it differed as to the readings recorded at the present level:

I The *Siwak* Group
II The Powdered *Siwak* Group
III The Starch Group
IV The Commercial Powder Group

TABLE I

PLAQUE PERCENTAGE

Material Used	1st week Mean ±S.D	2nd week Mean ±S.D	3rd week Mean ±S.D.	4th week Mean ±S.D.	5th week Mean ±S.D.
Siwak	41.7 ± 5.0	45.6 ± 6.2	43.3 ± 4.8	40.5 ± 5.0	38.2 ± 5.3
Powdered Siwak	27.6 ± 8.4	23.5 ± 7.9	19.6 ± 5.6	17.1 ± 5.0	16.4 ± 5.0
Starch	69.8 ± 8.2	73.6 ± 7.2	78.1 ± 6.4	80.4 ± 5.7	84.7 ± 4.6
Commercial Powder	9.2 ± 1.9	7.9 ± 1.9	6.9 ± 3.1	6.3 ± 3.0	5.3 ± 3.2

* 0 = Normal gingiva

1 = Mild inflammation, slight colour changes, slight edema, no bleeding on probing

2 = Moderate inflammation, red, edema, glazed, bleeding on probing

3 = Severe inflammation, marked redness and edema, ulceration, tendency to spontaneous bleeding.

Scores are totalled and divided by 4 to determine the gingival index for each tooth. Totalling of all indices, divided by the number of teeth in the mouth and multiplied by 100 will give the gingival percentage score for the individual.

Siwak group showed an increased plaque percentage in the first and second week but there was a decrease in this percentage by the end of the fifth week to give a difference between that of the first week of - 3.50%.

Powdered *Siwak* showed a greater amount in the difference between first and fifth week scores (-11.20%) i.e. a greater ability of

this substance when mechanically applied in a proper manner to rid the mouth of bacterial plaque.

Starch gave the worst scores of plaque percentage since the start of the first week. This score kept rising to give a difference in reading betwen first and fifth week of +14.90% i.e. a greater aggregation of plaque in the group using this material. Commercial powder gave a low score of plaque percentage from the start and kept dropping in a similar pattern given by powdered *Siwak* but the values differed in both cases.

TABLE II

GINGIVITIS PERCENTAGE

Material Used	PRESTART Mean ±S.D.	1ST WEEK Mean ±S.D.	2ND WEEK Mean ±S.D.	3RD WEEK Mean ±S.D.	4TH WEEK Mean ±S.D.	5TH WEEK Mean ±S.D.
Siwak	16.8 ± 4.4	16.8 ± 4.4	12.3 ± 3.8	12.3 ± 3.8	7.5 ± 3.4	6.1 ± 2.2
Powdered Siwak	8.6 ± 2.9	8.6 ± 2.9	6.8 ± 2.1	6.8 ± 2.1	4.7 ± 1.5	3.9 ± 1.6
Starch	28.1 ± 4.6	48.6 ± 6.9	52.2 ± 6.9	59.1 ± 6.6	65.0 ± 7.7	70.5 ± 8.4
Commercial Powder	9.8 ± 3.8	9.8 ± 3.8	9.8 ± 3.8	18.0 ± 8.7	21.8 ± 11.5	24.6 ± 13.8

Gingivitis percentage scores were recorded lowest in the powdered *Siwak* group from the first week of the experimental period. All scores for the differerrent materials used kept dropping except those of starch and commercial powder group, which indicates a deterioration of the gingival condition of both groups (differences between scores of first and fifth week are 42.40% and 14.80% respectively to the worse side).

PLAQUE PERCENTAGE

The commercial powder gave the lowest readings in plaque percentage scores. The drop in the powdered *Siwak* group is greater than that of *Siwak* while starch group gave an even increasing pattern to reach higher at the end of the fifth week.

GINGIVITIS PERCENTAGE

Powdered *Siwak* group showed the least amount of gingivitis resulting from its use. On the contrary this group originally showed mild gingivitis that kept dropping to the end of the experimental period indicating a possible therapeutic effect of powdered *Siwak* when properly used by a good mechanical device. *Siwak* group showed a better decline than that of the commercial powder group although the latter showed a better debriding effect of the mouth as indicated by the plaque percentage.

The starch users were the worst affected due to adhering nature of starch.

There are other findings that are of noticeable importance in this inverstigation:

1. Some *Siwak* users found some difficulty to apply the device to the tongue side of both upper and lower teeth.
2. Powdered *Siwak* users were not so happy as to the taste of the material in a powder form.
3. Starch users were met with several difficulties concerning the sticky nature of the powder.
4. Commercial powder users felt fine in the early phase of the experimental period, but nearing the end several complained of burning sensations in their mouths and 5 cases presented with actual peeling of their mucous membrane.

DISCUSSION

Oral hygiene and patient motivation towards a clean mouth owe their birth to the teachings of Prophet Mohammed (ﷺ). Due to the repeated use of *Siwak* during the day, the users showed an unusually high level of oral cleanliness. It is a well known fact that plaque is formed immedialtely after meticulous toothbrushing and by the end of 24 hours the plaque is well on its way towards maturation and hence starts its deliterious effects on the gingiva[8].

Proper oral hygiene should be maintained through intensive instructions by the periodontist as well as by a great expenditure of time and dexterity on part of the patient. This item is self corrected in Moslems because *Siwak* users take *Siwak* as a device that should be used as part of their religious ritual regimen.

The results obtained in this investigation have proved that *Siwak* and other tree twigs[9] could act as an effective tool in removing soft oral deposits. It could be even used as an effective device in preventive dental programmes in mass populations. The indices used in this investigation were simple and adequate as they discriminated between experimental stages as well as between experimental groups.

Using starch is not quite accurate but it was meant to evaluate the degree by which *Siwak* and powdered *Siwak* could rid teeth of deposits as compared to the best abrasive viz. commerical powder.

It is noticed that the difference between first and fifth week of the mean score of plaque percentage for powdered *Siwak* is the highest (-11.2%) of all readings. This indicates that if powdered *Siwak* is used with a mechanically proper device i.e. tooth brush, it will give a great deal of oral cleanliness.

It has been reported that *Salvadora persica* contains substances that possess antibacterial properties. Some other components are astringents, detergents and abrasives[8]. These properties encourage some toothpaste laboratories to incorporate powdered stems and/ or root material of *Salvadora persica* in their products (Beckenham U.K. Sarakan Ltd.).

Although the commercial powder gave a high degree of efficiency in plaque removal yet its use over the experimental period gave a high score of gingivitis percentage within the group using the powder. It is time that plaque eradication is essential but this should not be on the expense of deletirious side effects on other tissues.

It could be concluded that *Siwak* and powdered *Siwak* are excellent tools for oral cleanliness. Because of its availability in this part of the world, being inexpensive and readily adopted by Moslems as part of their religious regimen, it is highly recommended in implementing a preventive dental health program in Islamic countries. Also, recommendations should be directed to manufacturers of toothpastes to include the powdered form of *Siwak* in a highly debriding sophisticated toothpaste.

ACKNOWLEDGEMENT

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Our thanks are due to Dr. M. Salhieh, Associate Professor, Faculty of Art, Kuwait University, whose valuable help is greatly appreciated.

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PRELIMINARY CHEMICAL AND PHARMACOLOGICAL STUDY ON ALHAGI MANNIFERA

Drs. M.Th. Ghoneim, A.R. El-Gindy, R. Almi, E. Shoukry and R. Fatthouh

KUWAIT

PRELIMINARY CHEMICAL AND PHARMACOLOGICAL STUDY ON ALHAGI MANNIFERA*

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Abstract

Some preliminary pharmacological studies were carried out and it was found that the alcoholic extract of *Alhagi* produced a potent antispasmodic effect. In addition, the extract showed some histamine and serotonin antagonizing effects. It is suggested that the extract contains more than one ingredient.

INTRODUCTION

Alhagi mannifera (Desr) plants grow wild in Kuwait. It is used by the Beduins for treatment of renal colic. Hussein[7] reported that the extract of species grown in Egypt has a bronchodilator and, in addition, smooth muscle relaxant effect.

In the Phytochemistry Research Lab a preliminary chemical screening was done and found that it contains cardeneloids, sterols and triterpens, carbohydrates and/or glycosides, flavonoids but no alkaloid.

It was found of interest to study the possible pharmacological effects of this plant.

MATERIALS AND METHODS

A. The extracts of the plant.

1. The alcoholic extract of *Alhagi mannifera* Desr (AE) was prepared after successive extraction.

* Bulletin of Islamic Medicine, 1: 488 - 492, 1981.

2. The supernatant fraction of this alcoholic extract was prepared by diluting the extract with water (ranging from 1:5 to 1:100) then centrifuged. The supernantant portion was taken as a fraction (SAE).

3. The alcoholic extract of the powder was packed on silica gel and extracted by different organic solvents starting from petroleum spirit (40-60°), chloroform and then alcohol 95 per cent. Each extract was evaporated separately to dryness *in vacuo*. The residue was preserved in dessicator to be used as a fraction (AEAE).

B. The animals used included rabbits, albino rats, guinea pigs and toads of either sex. The animals were kept under the same conditions, allowed water *ad libitum*. The animals were kept fasting 12 hours before sacrifice.

C. Methods of pharmacological screening

The pharmacological investigations were carried out using the following preparations:

1. Isolated rabbits duodenum and jejunum (Modified Magnus technique, 1904)
2. Isolated guinea pig ileum (Burn, 1952 a)
3. Isolated skeletal muscle of the toad "rectus abdominus muscle" (Burn, 1952 b)
4. Isolated perfused rabbit's heart (Langendorff, 1895)
5. Isolated perfused toad's heart (Burn, 1952 c)
6. Isolated rat stomach fundus strip (Vane, 1957)
7. Isolated rabbit aortic strip (Furchgott & Bhadrakom, 1953)
8. Isolated guinea pig tracheal strip (Ghosh, 1971)
9. Isolated non-pregnant rat uterus (De Jalon, 1945)

RESULTS

The alcoholic extract (AE) of *Alhagi* produced a potent inhibitory effect on the tone and rhythmic activity of the rabbit's duodenum and jejunum (Fig. 1). The AE showed a potent inhibitory effect on the response of the muscle to angiotensin. In addition, it was able to abolish the spasmogenic effect of angiotensin (Fig. 2). The AE also inhibited the response of the muscle to serotonin (Fig. 3) but not to acetylcholine. The effect was reversible and dose-dependent. The AE reduced the response of the guinea pig tracheal strip to histamine (Fig. 4). It also inhibited the response of the rat uterus to the spasmogenic effect of angiotensin (Fig. 5). The AE reduced the response of the rat stomach fundus to serotonin induced contractions (Fig. 6). The AE caused relaxation of the guinea pig ileum when the muscle was under the spasmogenic effect of angiotensin (Fig. 7). The AE produced a stimulant effect on the isolated perfused heart, the effect was of a short duration. Large doses produced a stimulant effect which was followed by cardiac depression (Fig. 8). The AE produced a stimulant effect on the isolated toad's heart, mainly on the amplitude of contraction (Fig. 9).

The supernatant fraction (SAE) produced effects similar to those produced by the AE on the tone and rhythmic activity on the rabbit's duodenum and jejunum (Fig 10) and on the effects of angiotensin on the muscle (Fig 11). The SAE slightly reduced the response of the muscle to acetylcholine (Fig 12). On the guinea pig trachea, the SAE reduced the response of the muscle to the effect of histamine (Fig. 13) and serotonin (Fig. 14). The SAE decreased the response of the rat stomach fundus to serotonin (Fig. 15). On the guinea pig ileum, the SAE produced a stimulant effect by its own action. The effect was not abolished after atropine (Fig. 16a) or concentrated nicotine (Fig. 16b). The stimulation

was abolished after mepyramine, after this the SAE was able to decrease the response of the guinea pig ileum to angiotensin (Fig. 17). On the mammalian heart, the SAE produced a stimulant effect which was preceded by a short period of depression (Fig. 18). The stimulation slightly decreased after *beta*-adrenergic blockade (Fig. 19). The alcoholic eluate of the alcoholic extract (AEAE) of *Alhagi* was shown to produce inhibitory effect on the rabbit's duodenum and jejunum (Fig 20). The AEAE inhibited the response of the muscle to the effect of histamine (Fig. 21) and serotonin (Fig. 22) but not of acetylcholine. The AEAE decreased the spasmogenic effect of angiotensin (Fig. 23 a). The AEAE was able to relax the muscle which was under the effect of angiotensin (Fig. 23 b). The AEAE inhibited the response of the guinea pig tracheal strip to the effect of histamine (Fig 24).

DISCUSSION

The alcoholic extract of *Alhagi* was found to possess potent antispasmodic effect which was manifested on several preparations. The extract was shown to decrease the spasmogenic effect of angiotensin, this effect was clarified in 2 ways. Firstly, the extract decreased the response of the rabbit's duodenum to the spasmogenic effect of angiotensin. Secondly, the extract produced relaxation of the muscle which was under the spasmogenic effect of angiotensin. The extract also inhibited the spasmogenic effect of angiotensin on several other preparations. The spasmolytic effect of the extract is suggested to be mediated mainly through a direct effect on the smooth muscles since the extract was nearly devoid of anticholinergic or ganglion blocking effects. The extract was shown to exert histamine and serotonin antagonizing effects. On the guinea pig ileum, the supernatant fraction was shown to possess dual effects, the first is stimulatory and the second is inhibitory. The

inhibitory effect was masked by the influence of the stimulatory effect. The stimulation is not due to ganglionic or muscarinic effect but most probably mediated through a histamine like action because it was abolished after mepyramine. Once the stimulation was blocked by mepyramine, the supernatant fraction exerted its inhibitory effect which is suggested to be due to a direct action, this was manifested from the antagonizing effect to the spasmogenic action of angiotensin. Thus the supernatant fraction is believed to contain either one substance having a dual effect or more than one substance having different effects. The main pharmacological effects shown by the alcoholic extract were found to be also induced by the alcoholic eluate of the alcoholic extract. Other eluates were almost free from significant pharmacological actions. The alcoholic extract exerted moderate cardiac stimulant effect which is suggested to be due to a direct effect on the myocardium. This study is a preliminary one. Further investigations are required to be carried out to study the detailed pharmacological actions of the plant as well as the possible toxicity.

CONCLUSION

The alcoholic extract of *Alhagi* was shown to possess antispasmodic effect. The effect is suggested to be mediated through a direct action on the smooth muscles. The extract exerted some histamine and serotonin antagonizing effects on some smooth muscles. It is suggested that the extract contains more than one active ingredient.

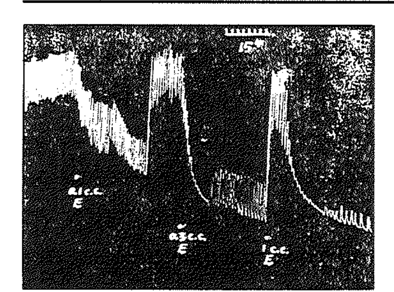


Fig. 1

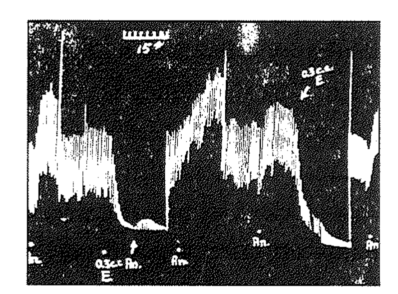


Fig. 2



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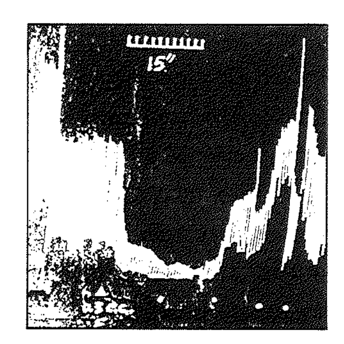


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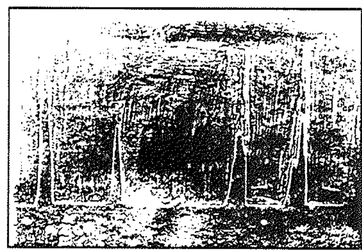


Fig. 6



Fig. 7

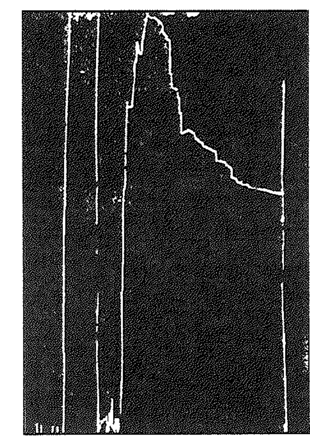


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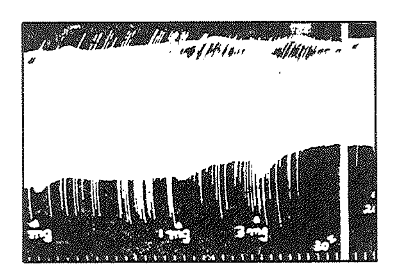


Fig. 9

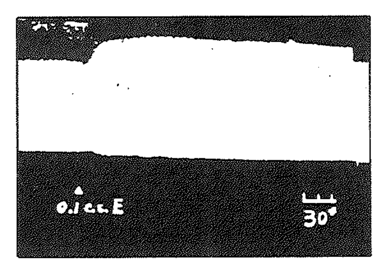


Fig. 10

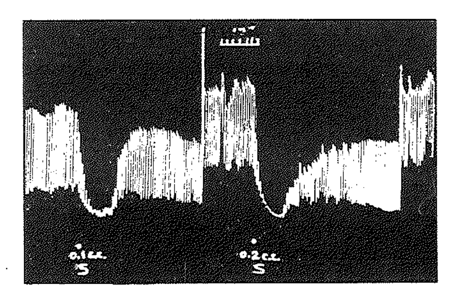


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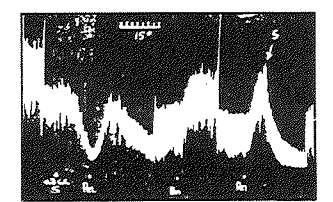
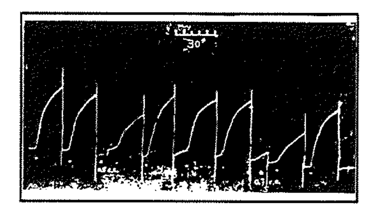


Fig. 12


Fig. 13

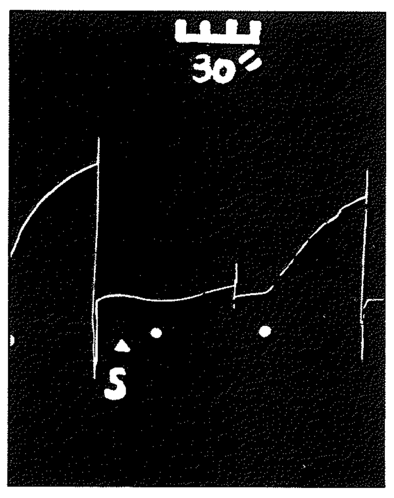
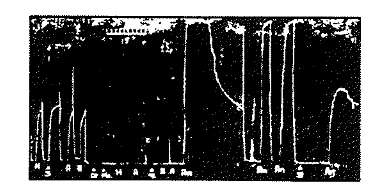


Fig. 14


Fig. 15


Fig. 16a


Fig. 16b


Fig. 17

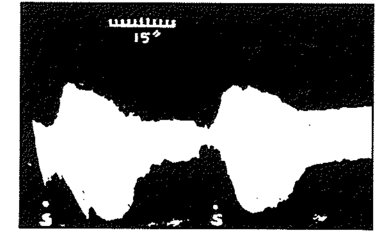


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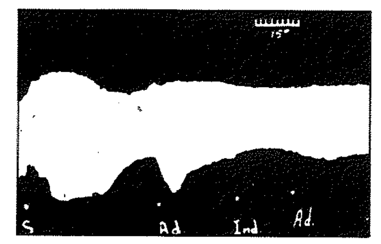


Fig. 19

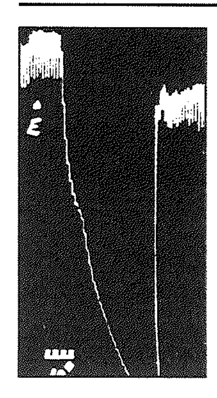


Fig. 20



Fig. 21

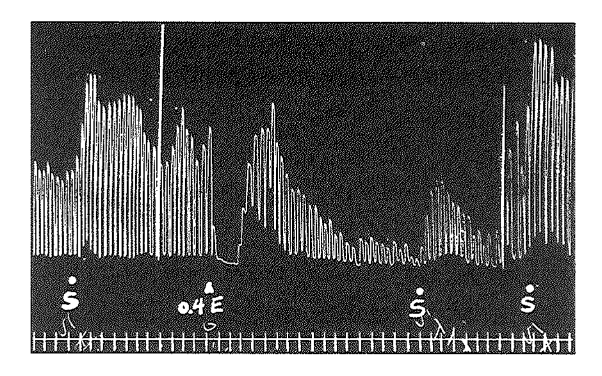


Fig. 22

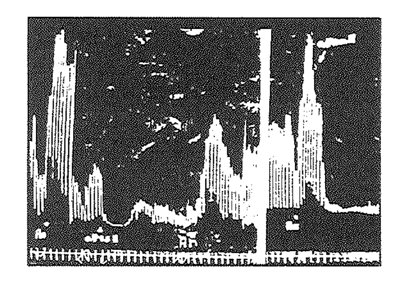


Fig. 23

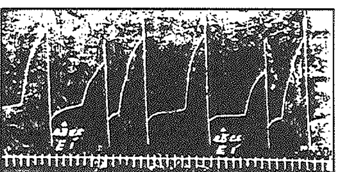


Fig. 24

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PRELIMINARY CHEMICAL AND PHARMACOLOGICAL STUDY OF ASTRAGALUS SPINOSUS GROWN IN KUWAIT

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KUWAIT

PRELIMINARY CHEMICAL AND PHARMACOLOGICAL STUDY OF ASTRAGALUS SPINOSUS GROWN IN KUWAIT*

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Abstract

Some preliminary pharmacological studies were carried out on the active ingredients and extracts obtained from *Astragalus spinosus* (Muschl). The glycoside was shown to possess positive inotropic properties as demonstrated from its effect on the isolated mammalian heart and rabbit atria. The alkaloidal fraction was found to produce a spasmolytic effect most probably through a direct action. The alkaloidal fraction showed moderate histamine and serotonin antagonizing effects. The alcoholic and chloroform extracts of the plant showed potent antispasmodic effect as shown from their effect on smooth muscles, this action is due to a direct effect on smooth muscle fibers. The alcoholic extract of the root showed some histamine and serotonin antagonizing effects.

The alcoholic extract of the shoots showed a similar effect as that produced by the glycoside. The chloroform extract of the shoots showed some antispasmodic effect in addition to some antihistaminic and antiserotonin effect.

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INTRODUCTION

The herbal treatment is an old method used by the mankind to cure ailments. There is no doubt that many of the important drugs of plant origin are still occupying its place in the medical treatment. The Islamic scientists were pioneers in such fields. Their prescriptions are still used up till now. So the field of herbal medicine is very important for further studies and research. *Astragalus spinosus* (Muschl) is a plant commonly used by the beduins in Kuwait and is known as *Shedad* for treatment of renal colic and bronchial asthma.

There are many species of *Astragalus* all over the world. The toxicity caused[1] by its ingestion by the livestock is divided into three groups(a) due to high percentage of selenium accumulated by the plant, (b) aliphatic nitro compounds, and (c) chronic true "loco" symptoms appearing particularly in horses. *Astragalus spinosus* (Muschl) grows in Egypt and Kuwait. Khaphaga *et al*[2] found that it contains glycoside but nothing is mentioned about its pharmacological action. So the aim of this work was to study its chemical compositions and to screen it pharmacologically.

EXPERIMENTAL

Materials and Methods

Chemical Study: *Astragalus spinosus* (Muschl) plants were collected during April. The shoots of the plants were dried in shade, powdered and packed in a continuous extraction apparatus. The powder was defatted, dried, extracted with chloroform and then with alcohol 95 per cent. From the chloroform extract a crystalline material "A" was isolated and an alkaloidal residue was obtained which on examination by TLC showed a single alkaloidal spot. But its quantity was scarce and trials to get more and study of its chemical properties will be dealt with later on. From the alcoholic extract a crystalline material "B" was obtained.

Isolation and Identification of Material "A"

The chloroform extract was concentrated and left in refrigerator. The material obtained was purified by silica gel column. Gradient elution was carried out, the ether fraction on concentration gave long needle shaped crystals and recrystallized from chloroform-light petroleum. It is insoluble in light petroleum, soluble in ether, chloroform, alcohol and water. The IR and NMR indicates that the material is acetamide.

Isolation and Identification of Material "B"

The alcoholic extract was concentrated and left at room temperature, white clusters of crystals were deposited. Recrystallization from pyridine water was done. The crystals were found to be organic in nature, m.p. 290-291° with decomposition. It is insoluble in water, chloroform, and ether. Soluble in pyridine, sparingly soluble in ethanol and methanol. Molecular wt 588. From UV, IR, MS and TLC the material was found to be aliphatic glycoside with arbinose as a sugar moity. The details of the chemical study is not dealt here to avoid a lengthy text and due to the limited time for its presentation.

The fractions were isolated from the plant according to the method described El-Gendi, *et al* [3]. The plant fractions include the following:

1. A glycoside
2. An alkaloidal fraction
3. The alcoholic extract of the shoots (AES)
4. The chloroform extract of the shoots (CES)
5. The alcoholic extract of the root (AER)
6. The chloroform extract of the root (CER)

Animals: The animals used in this study included rabbits, albino rats, guinea pigs, toads and cats of either sex. The animals were kept under the same conditions, allowed water *ad libitum*. The animals were kept fasting 12 hours before sacrifice.

Methods of screening:

1. Isolated rabbits' duodenum and jejunum (modified[4] Magnus technique, 1904)
2. Isolated guinea pig ileum (Burn, 1952 a[5])
3. Isolated skeletal muscle of the toad "rectus abdominis muscle" (Burn, 1952b[6]).
4. Isolated perfused rabbit's heart (Langendorff, 1895[7])
5. Isolated perfused toad's heart (Burn 1952 c[8])
6. Isolated rabbit's atria (Burn, 1952 d[9])
7. Isolated rat stomach fundus strip (Vane, 1957[10])
8. Isolated rabbit aortic strip (Furchgott & Bhadrakom, 1953[11])
9. Isolated guinea pig tracheal strip (Ghosh, 1971[12])
10. Isolated non-pregnant rat uterus (de Jalon, 1945[13])
11. Spinal cat blood pressure (Burn, 1952 e[8])

RESULTS

The glycoside produced only a slight inhibitory effect on the smooth muscles but had no significant effect on the skeletal muscle. On the isolated perfused mammalian heart, the glycoside in a small concentration produced a temporary depression followed by a gradual and slow stimulant effect mainly manifested on the amplitude of contraction. During the primary inhibitory phase, there was a slight decrease in the heart rate and coronary flow (Fig.

1). With large doses, the primary inhibitory phase lasted for longer duration and was accompanied by a decrease in the heart rate followed by cardiac arrhythmias and lastly the cardiac arrest. The stimulant effect of the glycoside was not abolished after β-adrenergic receptors blockade with propranolol (Fig. 2). Administration of the glycoside to hypodynamic heart (perfused with Ringer locke containing 2.5-12 μg quinine hydrochloride/ml) was found to decrease or prevent the induced hypodynamic state of the heart (Fig. 3). On the isolated rabbit's atria, the glycoside produced a stimulant effect mainly on the amplitude of contraction. The effect was slow to start (Fig. 4). The effect was not medicated through sympathetic stimulation (Fig. 5). On the toad's heart, the glycoside produced a stimulant effect mainly in the amplitude of contraction (Fig. 6). No significant effect was observed on the blood pressure of spinal cat. The alkaloidal fraction showed a mild inhibitory effect on rabbit jejunum (Fig. 7). It reduced the response of the guinea pig ileum to the effect of histamine (Fig. 8), serotonin (Fig. 9) and angiotension (Fig. 10) but not to the effect of acetylcholine. The alkaloidal fraction decreased the response of rat fundus stomach to serotonin (Fig. 11). The effect was dose-dependent. The response to angiotensin was also decreased but after a large concentration of the alkaloid (Fig. 12). The response to acetylcholine was not affected. On guinea pig trachea, the alkaloidal fraction reduced the response to histamine (Fig. 13). The effect was dose-dependent. On the rat uterus, the alkaloid produced a potent inhibitory effect on the response of the muscle to angiotensin (Fig. 14). No effect was observed on the skeletal muscle. On the mammalian heart, the alkaloidal fraction produced a stimulant effect mainly on the amplitude of contraction. The stimulation was not mediated through sympathetic stimulation (Fig. 15). The alcoholic extract of the shoots showed similar effects

to those produced by the glycoside. The chloroform extract of the shoot demonstrated effects more or less similar to those produced by the alkaloidal fraction. The alcoholic extract of the roots showed an inhibitory effect on the smooth muscles. The extract inhibited the tone and rhythmic activity of the rabbit duodenum and jejunum, the effect was dose-dependent (Fig. 16) and the muscles regained normal activity after washing of the drug. No significant effect was observed on the response of the muscle to acetylcholine, but the extract reduced the response to serotonin (Fig. 17) and abolished the response to barium chloride (Fig. 18).

On the guinea pig ileum, the extract inhibited the response of the muscle to contractions induced by histamine (Fig. 19), serotonin (Fig. 20) and angiotensin (Fig. 21). The response to nicotine was not affected except in very large concentration of the extract. The effect of the extract was dose-dependent and reversible. On the rat stomach fundus strip, the extract reduced the response to serotonin (Fig. 22). On the isolated guinea pig tracheal strip, the extract inhibited the response to angiotensin (Fig. 23) but not to histamine. On the isolated rabbit aortic strip, the extract reduced the response of the muscle to angiotensin (Fig. 24) but not to noradrenaline. On the mammalian heart, the extract produced a direct stimulant effect (Fig. 25). The chloroform extract of the roots was found to produce similar effect except that most of the effects produced on the smooth muscles were mediated through a direct inhibitory effect, where the extract was able to decrease the response of smooth muscles to angiotensin and barium chloride.

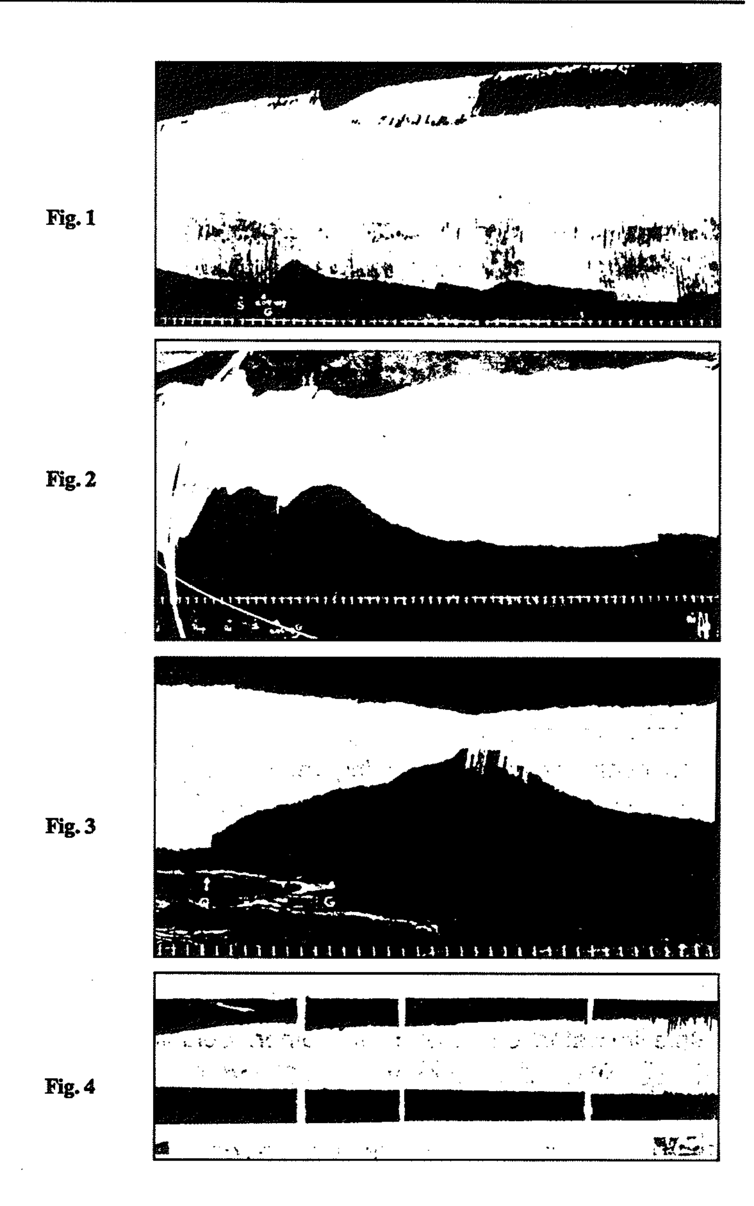


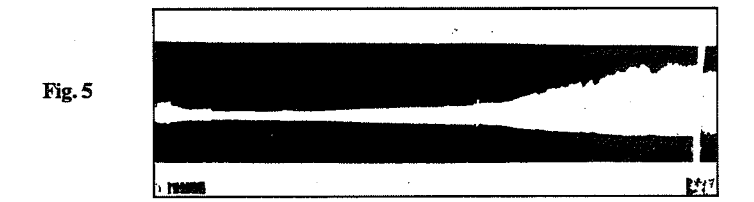
Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5



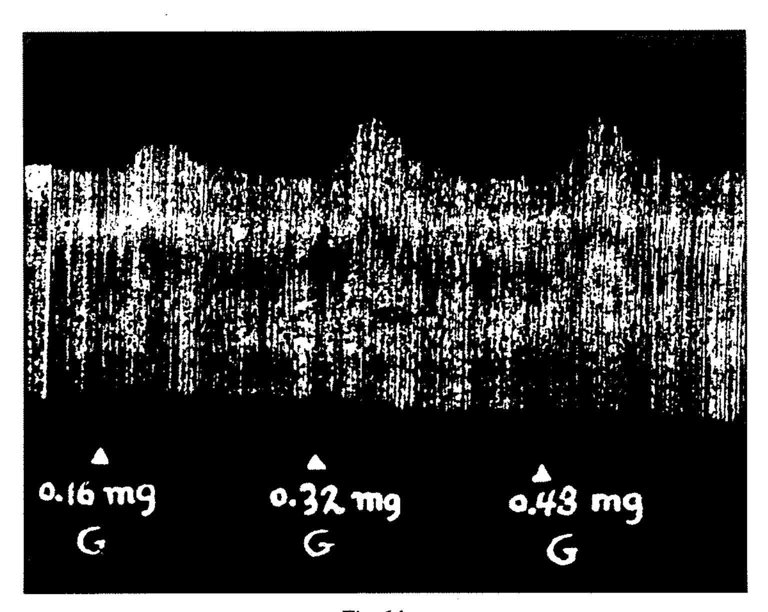


Fig. 6 ↑

Fig. 7



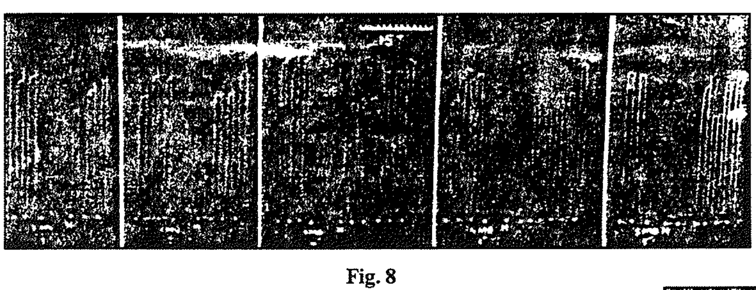


Fig. 8

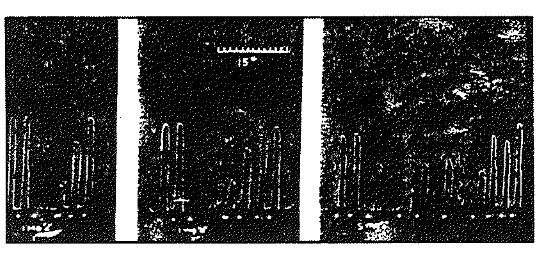
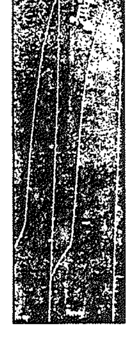


Fig. 9 ↑

Fig. 12 →



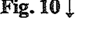
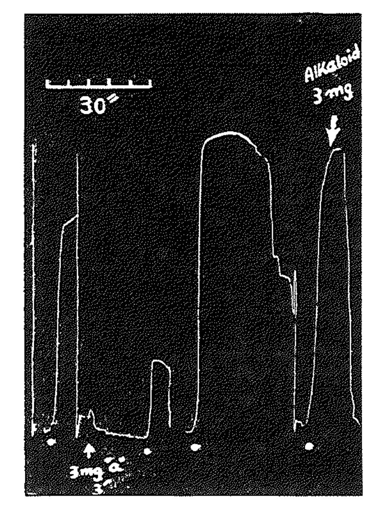
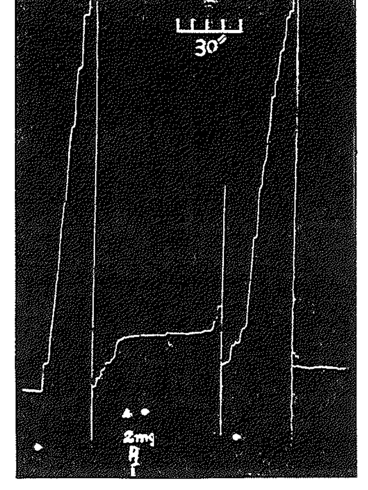


Fig. 10 ↓

Fig. 11 ↓





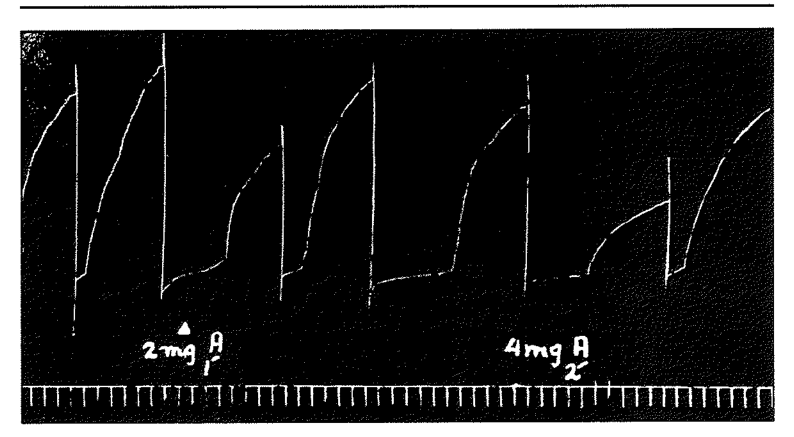


Fig. 13

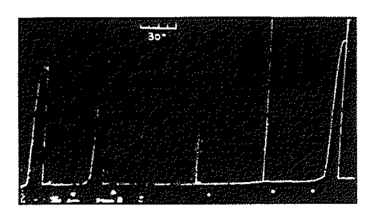


Fig. 14



Fig. 15

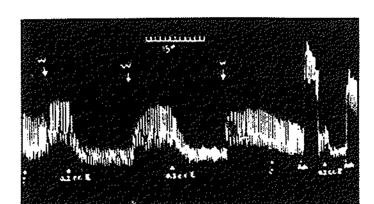


Fig. 16

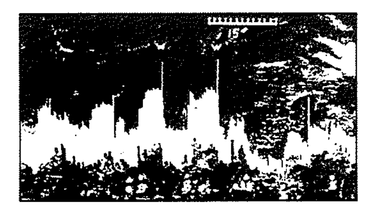


Fig. 17

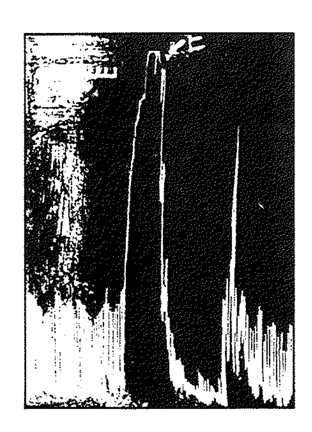


Fig. 18

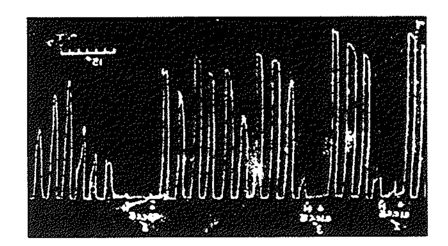


Fig. 19

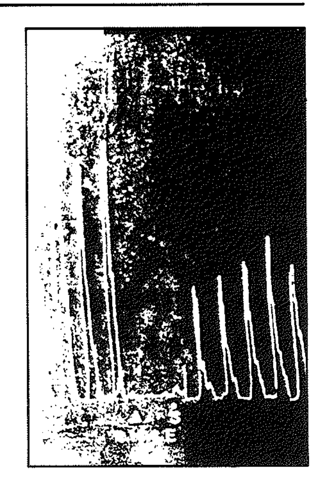


Fig. 20



Fig. 21

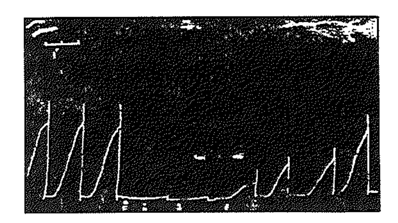


Fig. 22

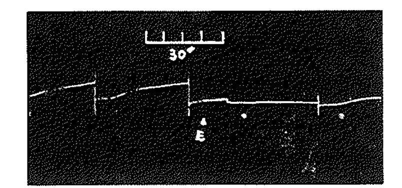


Fig. 23



Fig. 24



Fig. 25

DISCUSSION

Some pharmacolocical actions of the ingredients separated from *Astragalus*, in addition to the extracts, have been investigated. The glycoside is suggested to possess a positive inotropic effect on the heart as shown from its effect on the isolated mammalian heart. This preparation is sensitive to trace a cardiac glycoside like activity (Brown *et al*, 1962[14], Holland and Briggs, 1964[15]). The inotropic effect of the glycoside was manifested on both normal and hypodynamic hearts. It was reported that the degree of positive inotropic effect depends, to a great extent, on the functional state of the cardiac muscle (Sciarine *et al*, 1943[16], Braunwald *et al*, 1961[17], Rodman and Pastor, 1963[18]) where the effect is more pronounced on hypodynamic heart than in sufficient one. High concentrations of the glycoside were found to produce cardiac inhibition with a decrease in the heart rate, arrhyrthmias may occur and then the cardiac arrest. High concentrations of substances with cardiac glycoside like activity are known to impair the cardiac contractility, produce contracture in isolated hearts, the heart rate may be reduced and arrhythmias are likely to occur and finally the heart is functionally arrested (Klaus, 1966[19]). The glycoside was also shown to exert a positive inotropic effect on the isolated atria. Corresponding experiments for testing cardiac glycoside like activity were reported to be performed on isolated mammalian atria (Ehmer *et al*, 1964[20], Erjarec and Adamic, 1965[21], Greef *et al*, 1965[22]). The inotropic effect of the glycoside was not mediated through an adrenergic mechanism, most probably, it is mediated through a direct effect on the myocardium. Therapeutic actions of cardiac glycoside-like activity on the cardiac muscle are not mediated through adrenergic mechanisms and are not abolished by reserpine or adrenergic β-receptor blockers (Moran and Perkins, 1958[23], Morrow *et al*, 1963[24], Forster and Stolzenberg, 1963[25]). The glycoside is nearly devoid of significant antispasmodic effects.

The alkaloidal fraction produced an inhibitory effect on the smooth muscles. The ability of the alkaloidal fraction to inhibit the response to angiotensin indicates that it has a direct spasmolytic effect. In addition, the alkaloidal fraction showed moderate histamine and serotonin antogonizing effects. No significant anticholinergic effect was observed. The alkaloidal fraction showed a mild direct stimulant effect on the heart. The alcoholic and chloroform extract of the roots of *Astragalus* showed potent antispasmodic effect. The effect is not mediated through an anticholinergic effect. It is suggested that this effect is mediated through a direct action on the smooth muscles as proved from the antagonistic action to the spasmogenic effect of either angiotensin or barium chloride. The alcoholic extract showed some histamine and serotonin antogonizing effects. The alcoholic extract of the shoots of *Astragalus* showed a similar effect to that of the glycoside indicating that the glycoside is the main active principle present in this extract. The chloroform extract of the shoots of *Astragalus* also produced similar effects to those shown by the alkaloidal fraction suggesting that the alkaloid may constitute its main active ingredient.

CONCLUSION

Astragalus contains some active ingredients having different pharmacological effects. The main action of the glycoside and the alchoholic extract of the shoots was a direct cardiac stimulant effect. The other fractions showed potent antispasmodic effect. This is a preliminary study. Now, work is in progress to determine the other possible pharmacological effects and toxicity of the plant.

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PRELIMINARY PHARMACOLOGICAL STUDY OF THE FLOWERS OF SPHAERANTHUS HIRTUS

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INTRODUCTION

Sphaeranthus hirtus L. (globe flower) is a herb that grows in different parts of the world specially in India and Pakistan. The herb has been reported to possess different therapeutic effects. The roots of the plant were used as stomachic and anthelmintic. The flowers were used as what is called blood purifier in skin diseases and in jaundice (Nadkarni, 1976)[1]. The flowers were reported to produce some beneficial effects when given alone or in combination with other plants such as Fumaria, Chirata and Tephrosia in boils, abscess, itching and skin eruptions. Also, the plant has been used in palpitation (Kabiruddin, 1929)[2]. The plant was mentioned by Avicenna (1037 A.D.)[3] to be very useful in chronic ulcers, joint diseases, convulsions, jaundice and cough.

It was considered of importance to investigate the possible pharmacological effects of the extract obtained from this plant.

MATERIALS AND METHODS

Extract of the plant (AE)

The aqueous extract of the flowers of *Sphaeranthus* was prepared according to the method adopted by Hakeems[4]. 10 gm of the dried flowers of *Sphaeranthus hirtus* was kept in 200 ml distilled water for 24

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hours, then extraction was completed by boiling the whole mixture to concentrate it to half of its original volume. The extract was freshly prepared and used for the experimental work (100 gm of the dry flowers yielded 28.67 gm of the dry aqueous extract).

Pharmacological Methods

a) The animals used included rabbits, albino rats, guinea pigs and toads of either sex. The animals were kept under the same conditions and given water *ad libitum*. The animals were kept fasting 12 hours before sacrifice.

b) The investigation of the pharmacological properties of the aqueous extract (AE) of *Sphaeranthus* was carried out using the following procedures:

   1. *Isolated perfused rabbit's heart* (Burn, 1952)[5]. The effect of the aqueous extract of *Sphaeranthus* (AE) was studied on the force of contraction as well as the rate of contraction. The effect was also examined on the coronary outflow using different doses of the AE. Doses of vasoppressin (0.1 unit) were also given to investigate the effect of AE on the coronary vasoconstriction induced by vasopressin. The results were statistically analysed.
   2. *Isolated rabbit auricles* (Shoepke and Shideman, 1960)[(6)]. The contractile amplitude was recorded. The increase in height of lines on the Kymograph after addition of the drug to the bath was measured and compared with the height before addition, in order to express the inotropic effect of the drug as a percentage of the height or amplitude before the influence of the drug. The chronotropic effect was expressed as the percentage increase in number of lines (heart beats) per unit time.
   3. *Isolated rabbit aortic strip* (Furchgott and Bhadrakom, 1953)[7].
   4. *Blood pressure of anaesthetised rabbit* (Ghosh 1971)[8].
   5. *Isolated perfused toad's heart* (Burn, 1952)[9]

6. *Isolated rabbit duodenun and jejunum*
7. *Isolated guinea pig ileum* (Turner, 1965)[10]
8. *Isolated guinea pig tracheal strip* (Ghosh, 1971)[11]
9. *Isolated rat jejunum* (Van Rossum and Ariens, 1959)[12]
10. *Isolated rat stomach fundus strip* (Vane, 1957)[13]
11. *Isolated guinea pig vas deferens* (Leach, 1956)[14]
12. *Isolated rectus abdominis muscle of the toad* (Burn, 1952)[15]

RESULTS

On the isolated mammalian heart, the AE in a dose of 1.53mg produced a slight and temporary depressant effect on the rate and force of contraction. This was followed by slight stimulation in amplitude of contraction. The effect was dose dependent (Fig.1). The AE was found to increase the coronary outflow. In a dose of 1.529 mg, the AE produced an increase in coronary outflow equivalent to 14.5% ($P > 0.05$). In a dose of 3.058mg, the AE increased the coronary outflow by 29.8% ($P < 0.01$). Administration of vasopressin (0.1 unit) reduced the coronary flow by 49.2% ($P < 0.001$). Administration of the AE in a dose of 3.058mg immediately after vasopressin caused a decrease in coronary outflow equivalent to 15.20% from control value ($P > 0.05$). When this was compared to vasopressin, there was highly significant increase in coronary flow equivalent to 66.7% from the vasopressin group ($P < 0.01$ when compared to vasopressin). Administration of vasopressin after the AE, produced only a non significant decrease in coronary outflow equivalent to 14.8% of control ($P > 0.05$) and when compared to vasopressin group, there was still an increase in coronary outflow equivalent to 67.6% of vasopressin group ($P < 0.05$; Table 1). The effect of the AE (dose of 3.058 mg) on coronary outflow persisted for more than 5 min; Fig.2 shows the effect of the AE in addition to that of vasopressin.

On the isolated rabbit auricles, the AE caused a decrease in the rate when given in concentration of 1.1486 and 2.2936 mg/ml bath

solution (P -0.01 and -0.05 respectively). In a concentration of 2.2936 mg/ml bath solution, the AE produced a statistically significant increase in contractility (P -0.05) (Table II, Fig. 3 & 4).

On the isolated toad's heart, the AE produced a temporary depressant effect that was followed by a slight increase in amplitude of contraction. The effect was dose dependent (Fig. 5). The stimulation was not mediated through sympathetic stimulation. On the isolated rabbit aortic strip, the AE did not alter the response of the aortic strip to adrenaline. It decreased the response of angiotensin (Fig. 6).

On the anaesthetized rabbit blood pressure, the AE produced a very slight effect. In doses up to 17.14mg/kg body weight, the AE did not alter the response to adrenaline (Fig. 7). The AE inhibited the response to histamine.

On the isolated rabbit duodenum and jejunum, the AE in a concentration of 0.5734mg/ml bath solution produced a primary stimulant effect that was followed by a long lasting gradual inhibition in the tone of the muscle. The AE inhibited the response of the muscle to histamine (Fig. 8) and serotonin (Fig. 9) and in large concentration it slightly inhibited the response to acetylcholine (Fig. 10).

On the guinea pig ileum, the AE in concentration of 1.4335 mg/ml bath firstly produced a stimulant effect of its own which was gradually abolished. In this concentration, the AE selectively inhibited the response of the muscle to histamine but not to acetylcholine (Fig. 11). Only in large concentrations it slightly reduced the response to acetylcholine (Fig. 12). The AE reduced the response of the muscle to serotonin (Fig. 13), angiotensin (Fig. 14) but not to nicotine (Fig. 15). The initial stimulation produced by the AE was abolished by atropine (Fig. 16). On the guinea pig trachea, the AE in a concentration of 1.7202mg/ml bath solution produced a potent relaxant effect; in addition, it strongly inhibited the

histamine induced contractions (Fig. 17). The effect was persistent for a long duration.

The AE in a concentration of 0.819 mg/ml bath solution reduced the response of rat fundus strip to serotonin (Fig. 18) and only slightly and temporarily to acetylcholine (Fig. 19).

On the isolated rat jejunum, the AE in a concentration of 1.1468 mg/ml bath solution produced relaxant effect. It strongly reduced the response of the muscle to angiotensin (Fig. 20). It slightly reduced the response to acetylcholine (Fig. 21).

The AE in concentrations of 0.7167-1.4335 mg/ml bath solution did not affect the response of guinea pig vas deferens to contractions induced by adrenaline (Fig. 22). The same concentration reduced the response to histamine but not to acetylcholine (Fig. 23).

On the rectus abdominis muscle, the AE showed only a slight potentiating effect to contraction induced by acetylcholine (Fig. 24).

DISCUSSION

The aqueous extract of *Sphaeranthus* was found to possess a spasmolytic effect on the smooth muscles.

The relaxant effect is suggested to be mediated through a direct action on the smooth muscles. This was shown from the inhibitory effect of the AE on the contractions induced by angiotensin on several preparations such as the guinea pig ileum, rat jejunum and isolated rabbit aortic strip. The AE was able to decrease the vasoconstrictor effect of vasopressin on coronary circulation. The inhibitory effect on smooth muscles induced by the AE is not mediated through a ganglion blocking effect because the AE did not alter the response of any of the smooth muscle tested to nicotine. The relaxant effect is also not related to adrenergic

stimulation. The AE did not affect the response of rabbit aortic strip or guinea pig vas deferens to adrenaline.

In several isolated smooth muscles, it was found that the AE produced a primary stimulant effect which was followed by an inhibitory action. The first effect was abolished by atropine suggesting that the AE may contain more than one active ingredient, one is a cholinergic stimulant and the other is an inhibitor. The AE was found to possess anti-histamine effect as well as antiserotonin effect. The AE inhibited the response of several isolated preparations to the contractions induced by histamine. It inhibited the effect of histamine on blood pressure. Preziosi (1958)[16] used the rabbit blood pressure for studies on antihistamines where diminished responses to histamine after administration of a drug indicates antihistaminic activity. Within certain concentrations, the effect of the AE was specific for antagonism of histamine and serotonin. In larger concentrations, the AE slightly reduced the response of some isolated preparations to acetylcholine suggesting that the AE may possess slight anticholinergic effect. The AE produced a statistically significant increase in the coronary outflow. This action is mostly due to a direct effect on the coronary circulation. The increase in coronory flow is not believed to be mediated through increase in the force of contraction. Doses of the AE that produced increase in the coronary flow did not show significant inotropic effect. Only large doses of the AE possess inotropic effect. The AE has some negative chronotropic effect. The effect of AE on coronary flow was first studied on normal circulation. Vasopressin is known to cause acute coronary vasoconstriction (Lindner *et al*, 1953)[17]. The AE was able to antagonize the vasoconstrictor effect of vasopressin on the coronary circulation. Vasopressin antagonism was reported repeatedly after administration of coronary dilating drugs in the Langendorff heart as well as in intact animals (Lindner *et al*, 1953)[17].

TABLE I

EFFECT OF AQUEOUS EXTRACT (AE) OF SPHAERANTHUS HIRTUS FLOWERS AND VASOPRESSIN ON CORONARY FLOW

	Control	Effect of 1.529 mg AE	Effect of 3.058 mg. AE	Effect of vasopressin (0.1U)	Effect of 3.058 mg AE after vaspressin (0.1 U)	Effect of vasopressin (0.1U) after 3.058mg AE
Mean ± S.E.	10.95[d] ± 0.454 (22)[a]	11.555 ± 1.250 (6)	13.104 ± 0.823 (16)	5.134 ± 1.085 (8)	8.558 ± 0.626 (10)	8.604 ±' 0.992 (9)
p^b_1	-	> 0.05	< 0.01	< 0.001	> 0.05	> 0.05
% change from control	-	+ 14.50%	+ 29.8%	- 49.2%	- 15.2%	- 14.8%
p^c_2	-	-	-	-	< 0.01	< 0.05
% change from vasopressin	-	-	-	-	+ 66.7%	+ 67.7%

(a) Number of animals

(b) as compared to control group

(c) as compared to vasopressin group

(d) Figures are the average of coronary flow in ml per mintue of 3 minutes after adminsitration of drug.

TABLE II

EFFECT OF AQUEOUS EXTRACT (AE) OF SPHAERANTHUS HIRTUS ON THE RATE AND FORCE OF CONTRACTION OF ISOLATED RABBIT'S AURICLES

	RATE[a]				FORCE OF CONTRACTION[b]			
	Control	Effect of 0.5473 mg AE	Effect of 1.1486 mg AE	Effect of 2.2936 mg AE of sphaeranthus	Control	Effect of 0.5743 mg AE	Effect of 1.486 mg. AE	Effect of 2.2936 mg AE
Mean ± S.E.	102.42 ± 5.95 (12)[c]	97.80 ± 6.97 (10)	79.00 ± 4.79 (12)	81.88 ± 7.42 (8)	2.229 ± 0.223 (12)	2.172 ± 0.206 (10)	2.518 ± 0.339 (12)	3.385 ± 0.417 (8)
p	-	> 0.05	< 0.01	< 0.05	-	> 0.05	> 0.05	< 0.05
% change from control		- 4.50%	- 22.9%	-20.1%	-	-2.5%	+ 12.9%	+ 51.9%

(a) The rate is expressed as number of lines (heart beats) per unit time as recorded from the contractile amplitude of auricular contraction

(b) The force of contraction is expressed as the height of lines (in cm) recorded from the contractile amplitude of contraction (Schoepke and Shideman, 1960).

(c) Number of animals

The concentration of AE represents per ml bath solution.

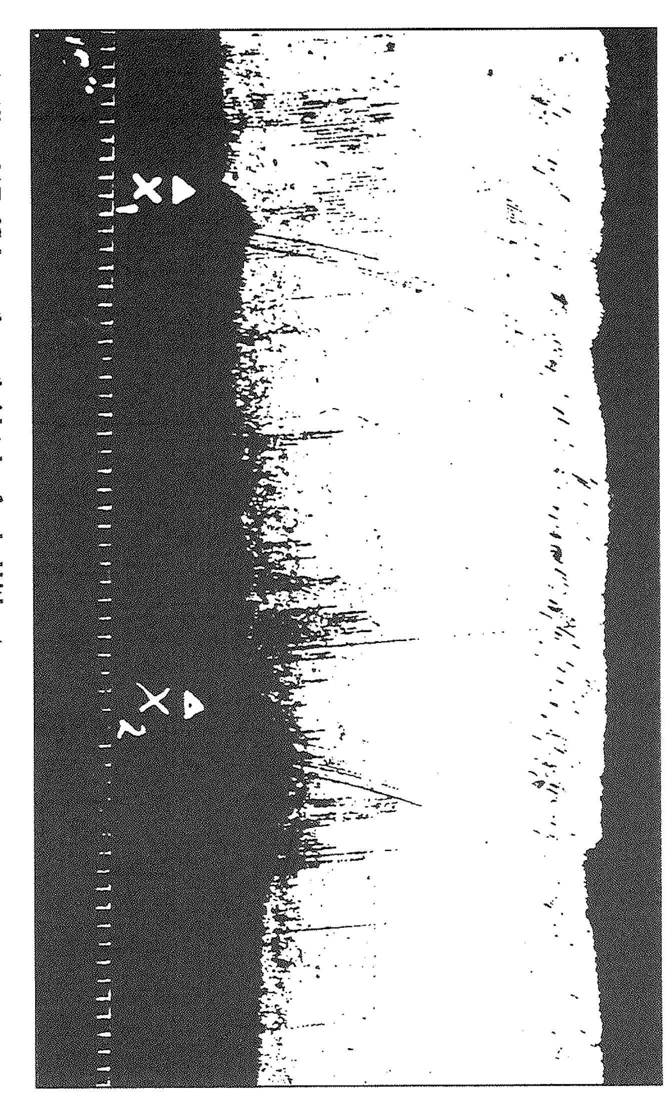


Fig. 1: Effect of AE of Sphaeranthus on the islated perfused rabbit heart.

X_1: 1.5290 mg AE; X_2: 3.0580 mg AE

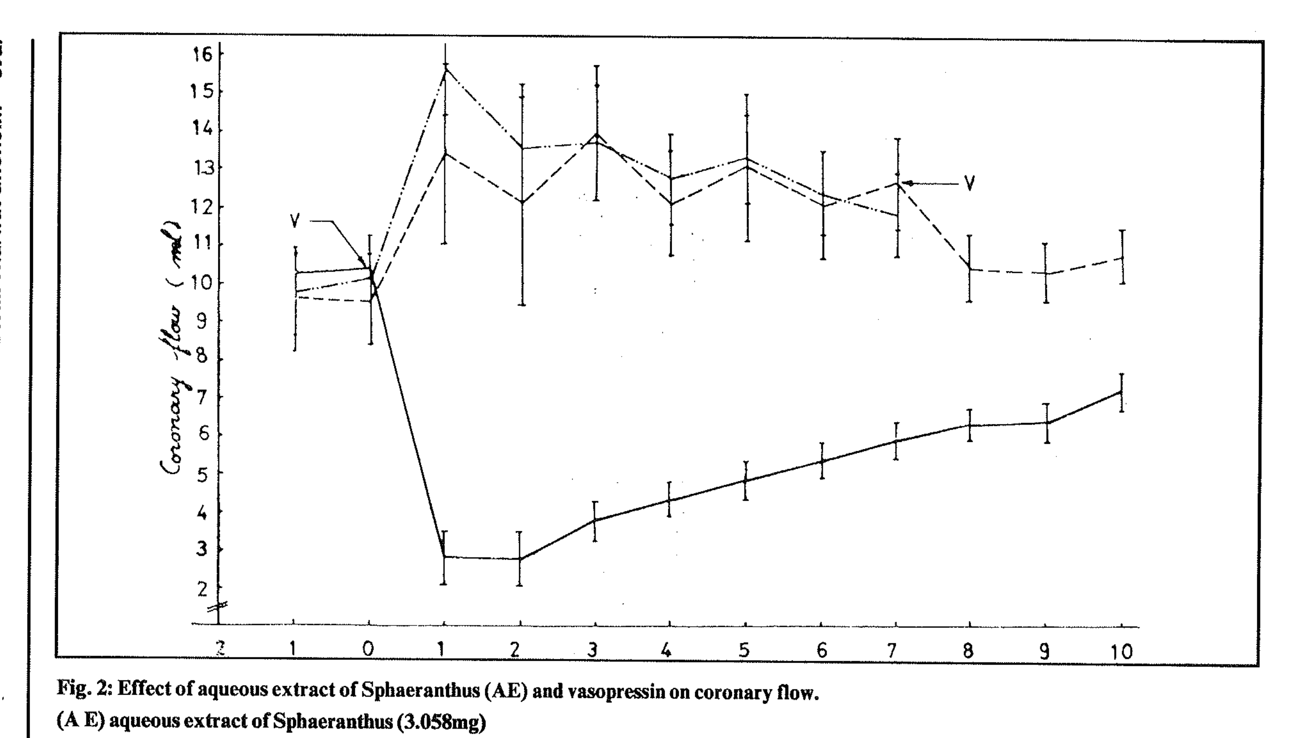


Fig. 2: Effect of aqueous extract of Sphaeranthus (AE) and vasopressin on coronary flow.

(A E) aqueous extract of Sphaeranthus (3.058mg)

Vasopressin (0.1U)

(AE) followed by vasopressin

Rate ———
Force -·-·-·-

160
150
140
130
120
110
100
90
80
70
1.0

Percent from control

0.5 1.0 1.5 2.0

0.5743 1.1486 1.7229 2.2936

mg AE / ml bath solution

Fig. 3: The effect of AE of Sphaeranthus on isolated rabbit's auricles. The effect is on the rate and force of contraction.

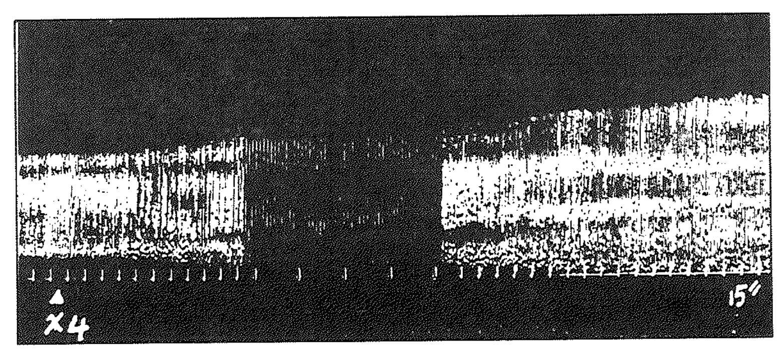
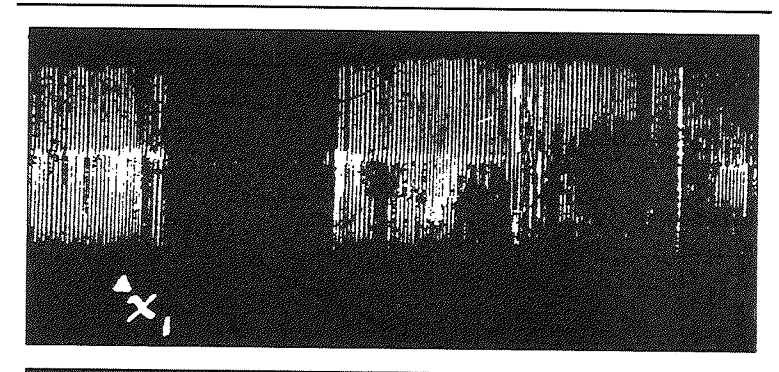
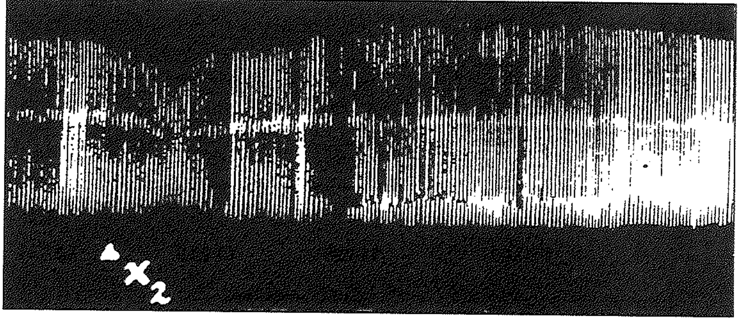


Fig. 4: Effect of AE of Sphaeranthus on isolated rabbit auricles.
X_4: 1.1468 mg AE / ml bath solution





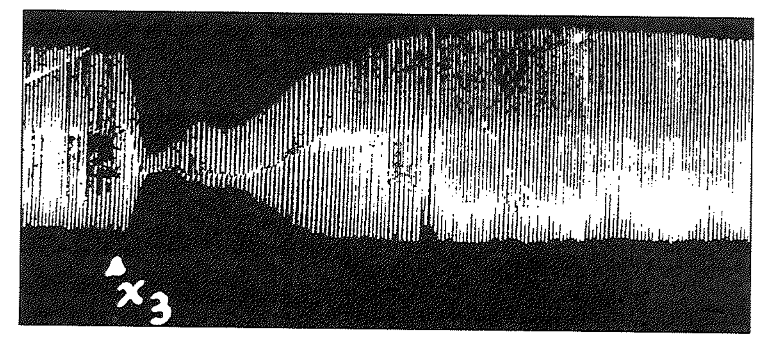


Fig. 5: Effect of AE of Sphaeranthus on isolated perfused toad's heart.

X_1: 2.867 mg AE

X_2: 5.734 mg AE

X_3: 8.601 mg AE

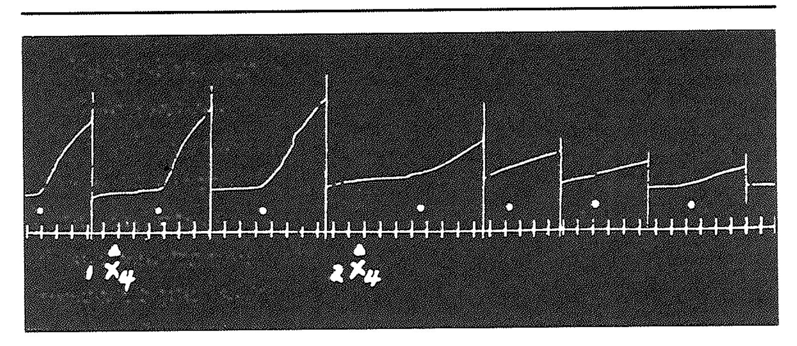


Fig. 6: Effect of AE of Sphaeranthus on isolated rabbit aortic strip.
1 x 4: 0.1433mg AE / ml bath solution
2 x 4: 0.2867 AE / ml bath solution
Unmarked contractions are due to angiotensin.

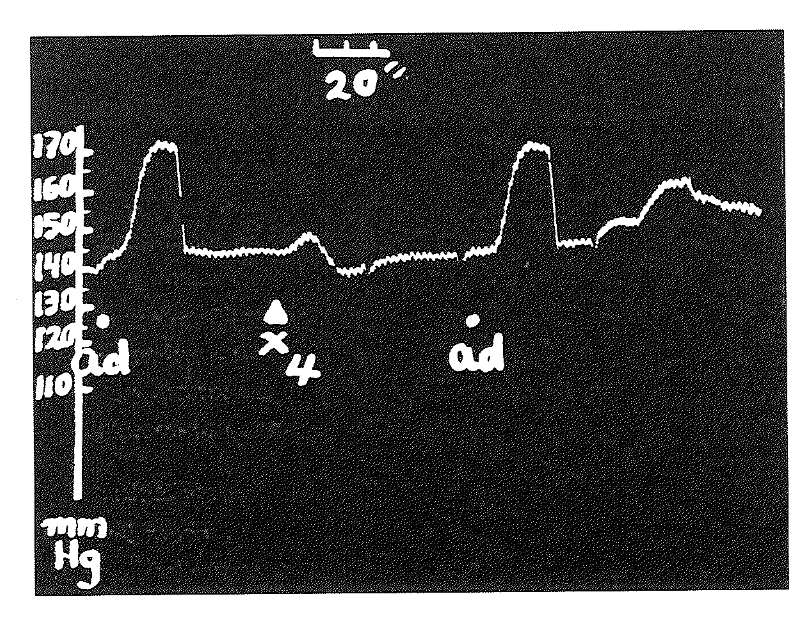


Fig. 7: Effect of AE of Sphaeranthus on rabbit. Rabbit (1.75kg), anaesthetized with urethane (1gm/kg)
X_4: 17. 14mg AE/kg i.v; ad: adrenaline

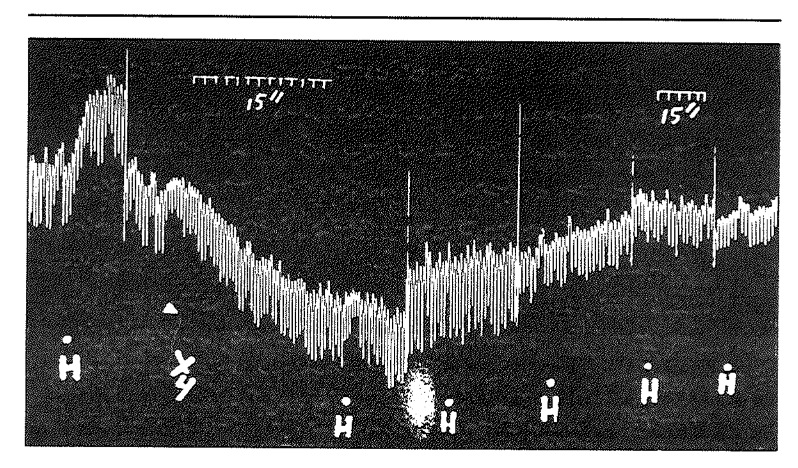
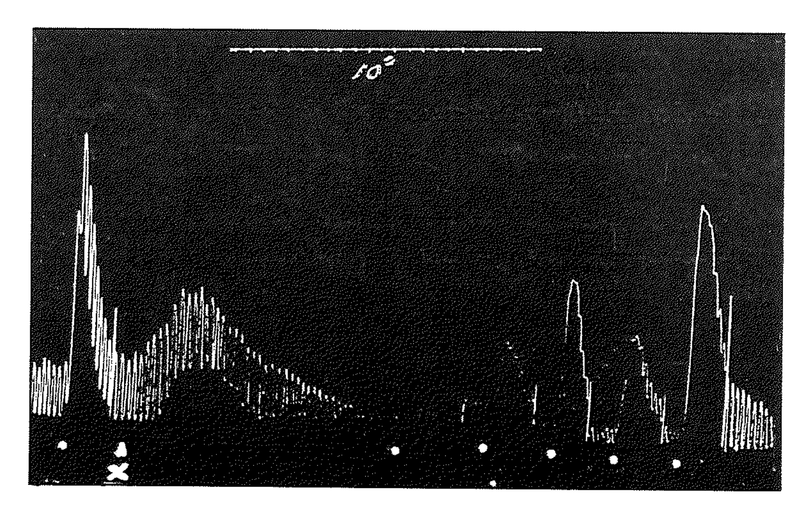


Fig. 8: Effect of aqueous extract (AE) of Sphaeranthus on isolated rabbit duodenum
H: Histamine; X_4: 0.5734 AE/ml bath solution



Fg. 9: Effect of aqueous extract (AE) of Sphaeranthus on isolated rabbit duodenum. Unmarked contractions are due to serotonin; x: 1.1468 mg AE / ml bath solution

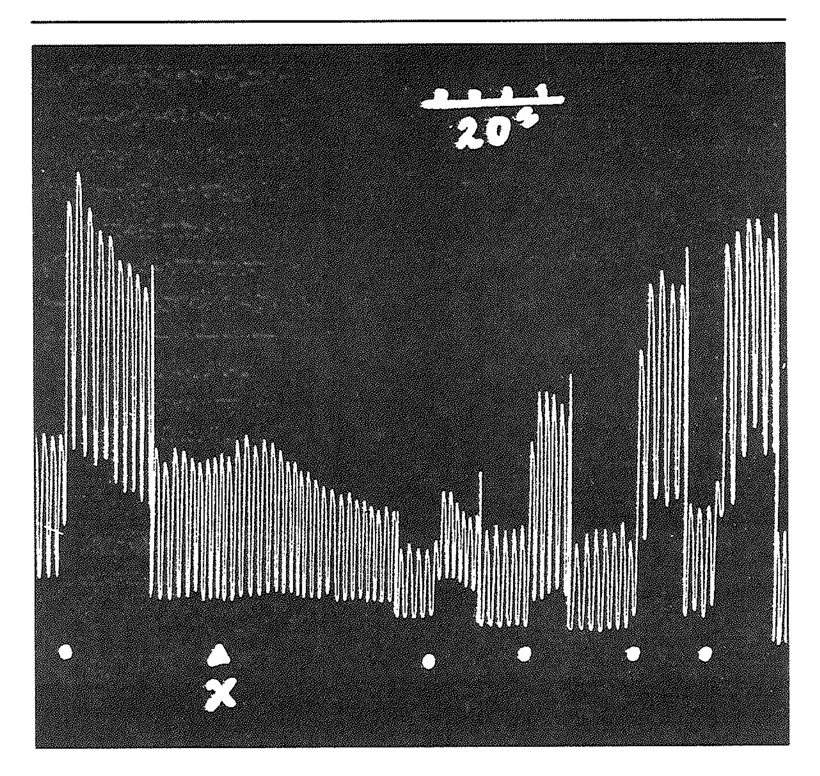


Fig. 10: Effect of aqueous extract (AE) of Sphaeranthus on isolated rabbit duodenum. Unmarked contractions are due to acetylcholine; x: 2.2948mg AE / ml bath solution

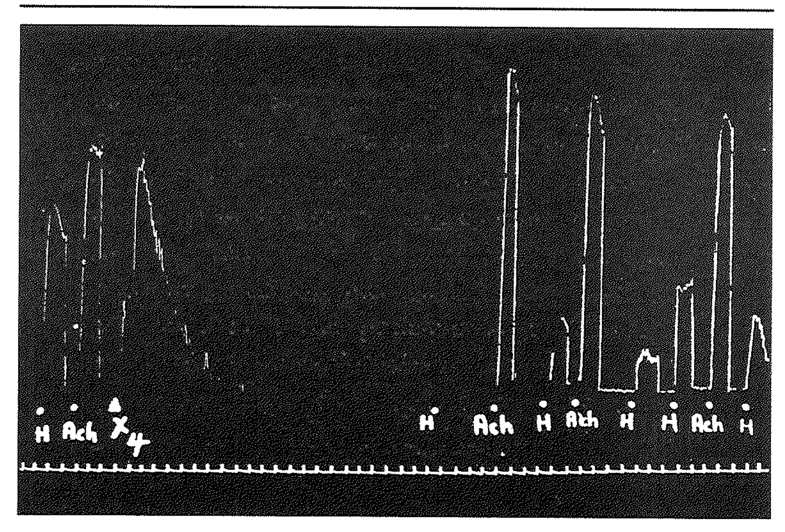


Fig. 11: Effect of aqueous extract (AE) of Sphaeranthus on guinea pig ileum.
H: Histamine; X_4: 1.4335 mg AE / ml bath solution; Ach: Acetylcholine

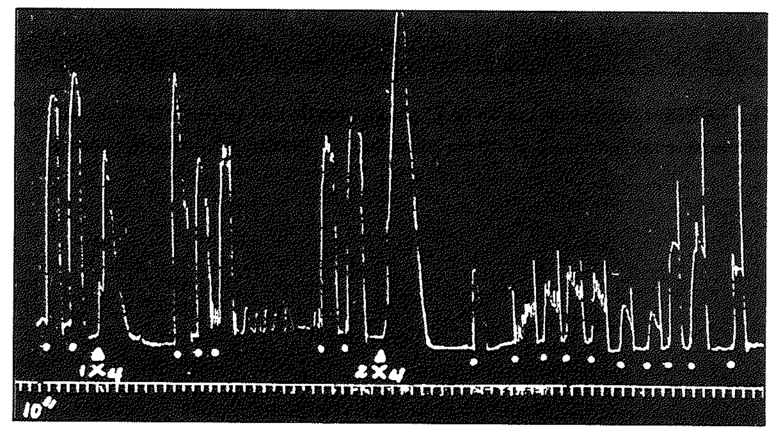


Fig. 12: Effect of aqueous extract (AE) of Sphaeranthus on guinea pig ileum.
Unmarked contractions are due to acetylcholine
1 x 4: 1.1468 mg AE / ml bath solution
2 x 4:2.2936 mg AE / ml bath solution

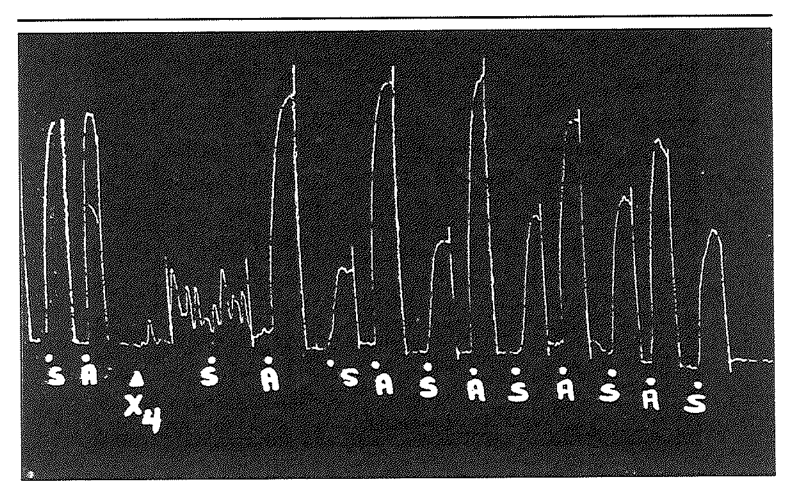


Fig. 13: Effect of aqueous extract (AE) of Sphaeranthus on guinea pig ileum
S: Serotonin; A: Acetylcholine; X_4: 0.5734 mg AE / ml bath solution

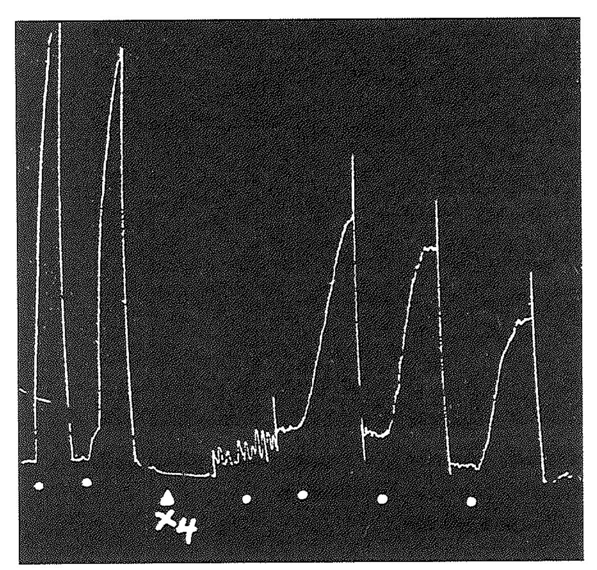


Fig. 14: Effect of aqueous extract (AE) of Sphaeranthus on guinea pig ileum
Unmarked contractions are due to angiotensin
X_4: 0.5734 mg AE / ml bath solution

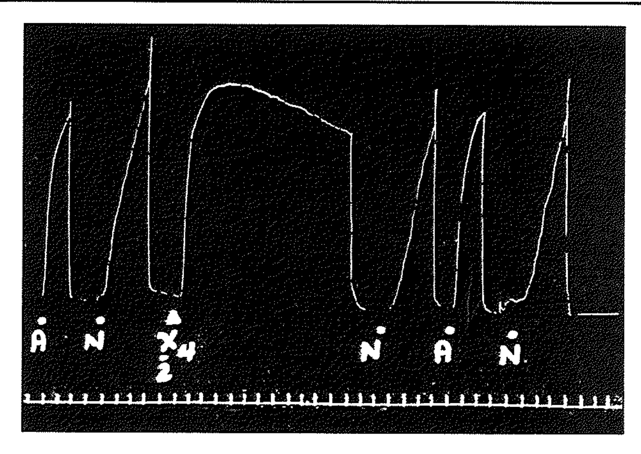


Fig. 15: Effect of aqueous extract (AE) of Sphaeranthus on guinea pig ileum
N: nicotine; A: Acetylcholine
X_4: 0.71675 mg AE / ml bath solution

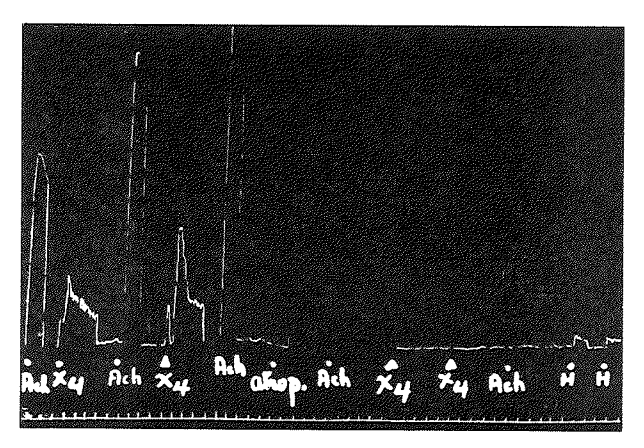


Fig. 16: Effect of aqueous extract (AE) of Sphaeranthus on guinea pig ileum
Ach: acetylcholine; Atrop: Atropine; H: Histamine
X_4: 1.4335 mg AE / ml bath solution

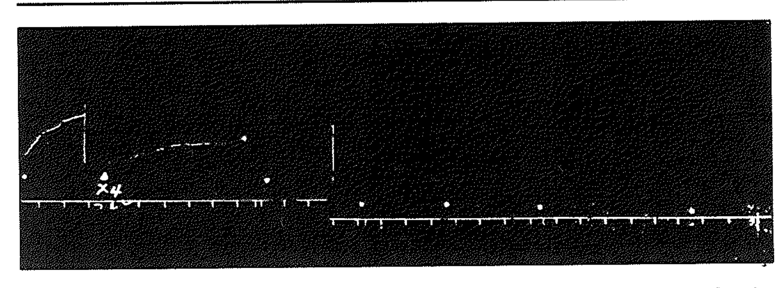


Fig. 17: Effect of aqueous extract (AE) of Sphaeranthus on guinea pig tracheal strip. Unmarked contractions are due to histamine; X_4: 1.7202mg AE / ml bath solution

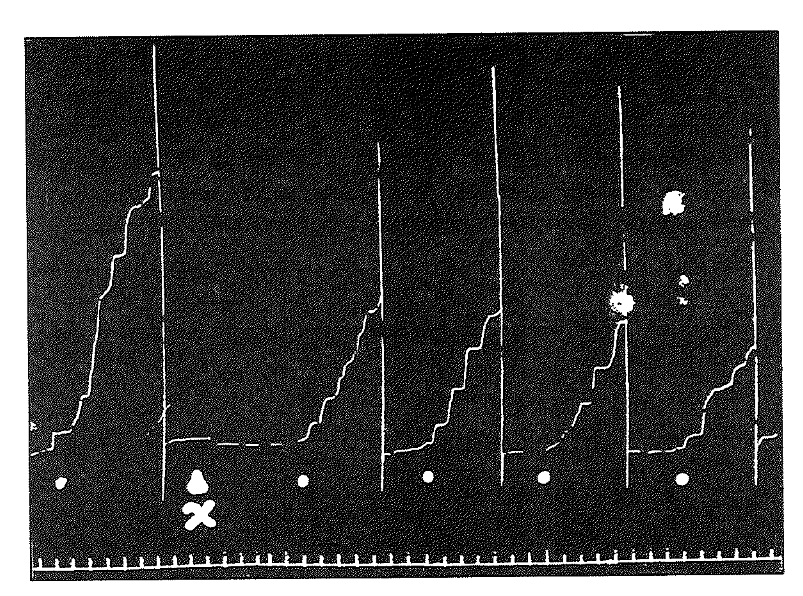


Fig. 18: Effect of aqueous extract (AE) of Sphaeranthus on rat stomach fundus. Unmarked contractions are due to serotonin; x: 0.819 mg AE / ml bath solution

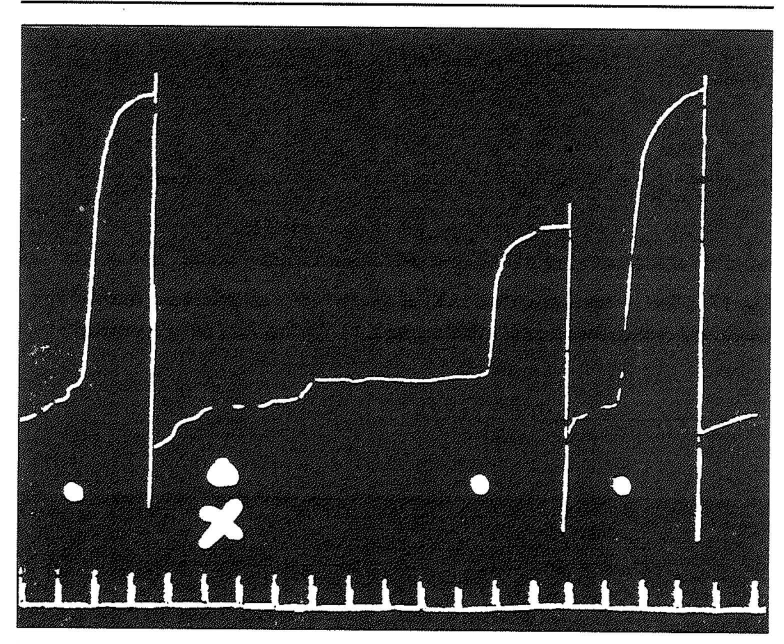


Fig. 19: Effect of aqueous extract (AE) of Sphaeranthus on rat stomach fundus strip. Unmarked contractions are due to acetylcholine; x: 0.819mg AE / ml bath solution

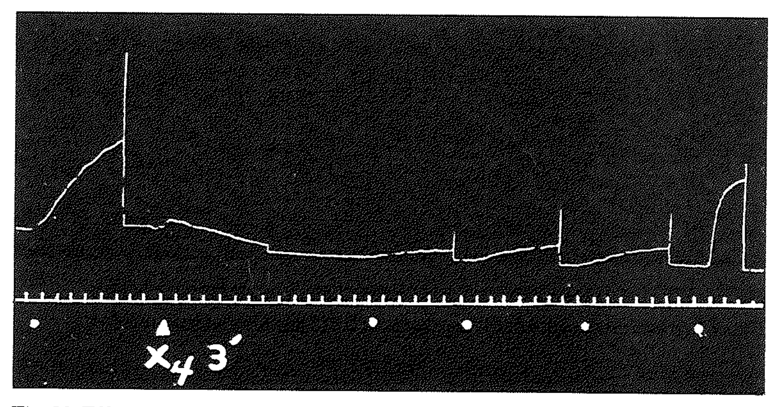


Fig. 20: Effect of aqueous extract (AE) of Sphaeranthus on isolated rat jejunum. Unmarked contractions are due to angiotensin; X_4: 1.1468 mg AE / ml bath solution

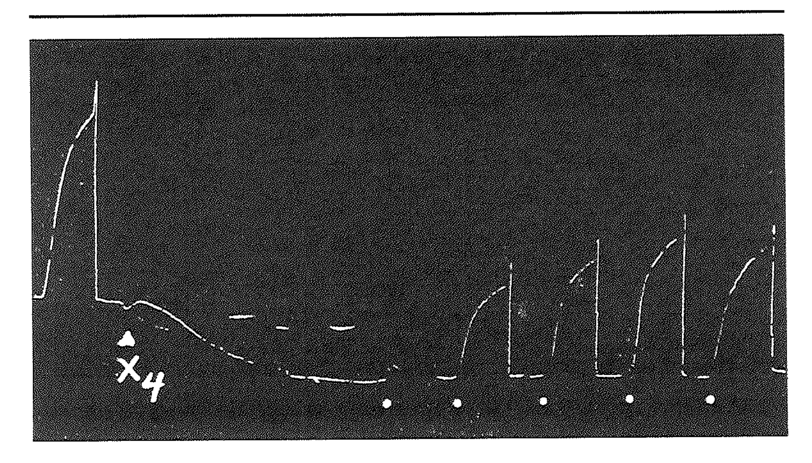


Fig. 21: Effect of aqueous extract (AE) of Sphaeranthus on isolated rat jejunum.
Unmarked contractions are due to acetylcholine; X: 1.1468mg AE / ml bath solution

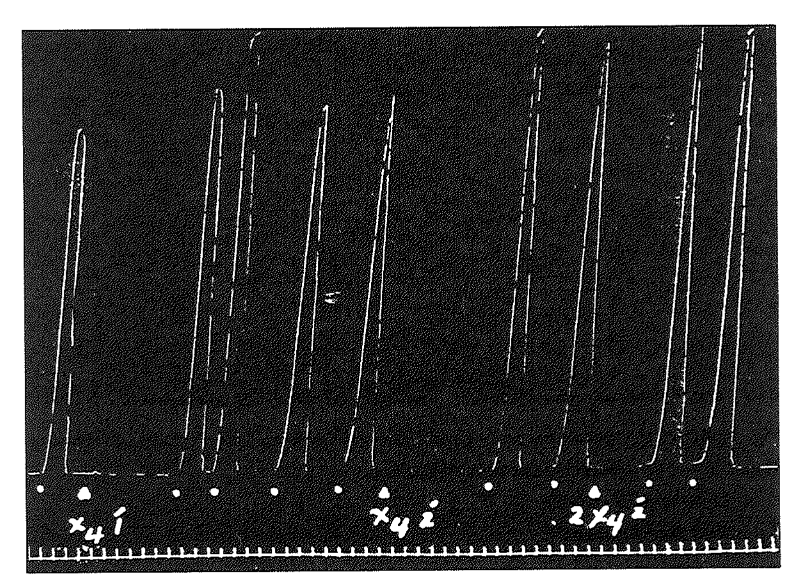


Fig. 22: Effect of aqueous extract (AE) of Sphaeranthus on isolated guinea pig vas deferens.
Unmarked contractions are due to adrenaline.
X4: 0.7168 AE/ ml bath solution.
2 x 4: 1.4335 mg AE / ml bath solution

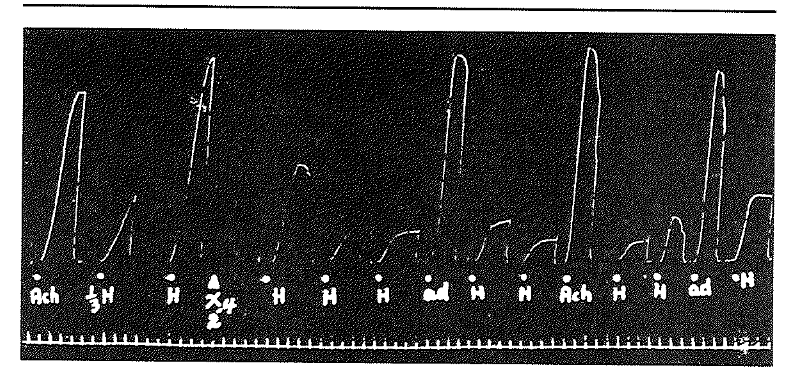


Fig. 23: Effect of aqueous extract (AE) of Sphaeranthus on isolated guinea pig vas deferens.
H: Histamine; Ach: Acetylcholine; ad: adrenaline
X_4: 1.4335 mg AE / ml bath solution

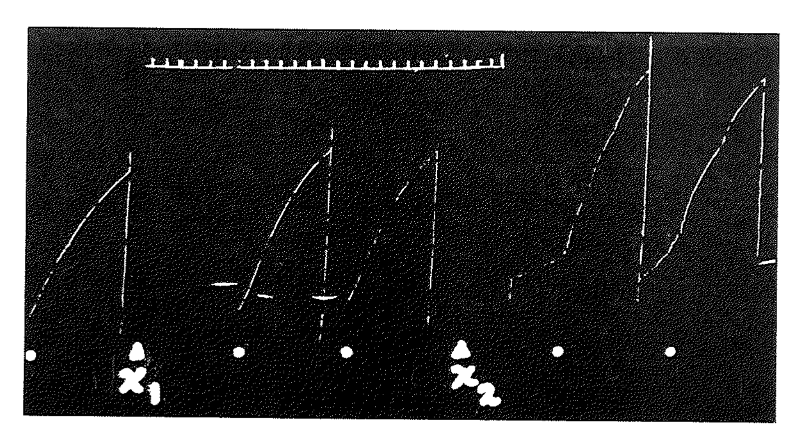


Fig. 24: Effect of aqueous extract (AE) of Sphaeranthus on toad rectus abdominis muscle.
Unmarked contractions are due to acetylcholine.
X_1: 0.7168 mg AE / ml bath
X_2: 1.4335 mg AE / ml bath

The AE produced a slight non-significant effect on blood pressure.

It is concluded that the AE contains one or more active ingredient(s) which possess antihistamine and antiserotonin effects in addition to a bronchodilator and smooth muscle relaxant effect. The AE also showed a coronary vasolidator effect.

This is only a preliminary report. Further investigations are required to explore the chemical composition of this plant in order to determine the exact pharmacological effect of any possible active ingredient that may be present in this plant. Also toxicity studies for acute and chronic administration of the AE and the active ingredient is required. The preliminary data obtained in this work are encouraging.

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CONTINUED USE OF IRRITANT AND CO-CARCINOGENIC EUPHORBIACEAE PLANTS IN ISLAMIC MEDICINE

Dr. Gulam Abbas Miana

PAKISTAN

CONTINUED USE OF IRRITANT AND CO-CARCINOGENIC EUPHORBIACEAE PLANTS IN ISLAMIC MEDICINE*

Dr. Gulam Abbas Miana
PAKISTAN

Many plants belonging to the family Euphorbiaceae are being used in the indigenous health care system of most of the Islamic countries of the world. Glossary of Indian Medicinal Plants[1] lists at least 50 such species (Tale I). Prominent among these plants are the following:

1. *Croton tiglium*
2. *Mallotus philippinensis*
3. *Euphorbia resinifera*
4. *Emblica officinalis*

Seeds of *Croton tiglium* and its oil have been used[1] as purgative and as counter-irritant. Glands and hairs on the fruit of *Mallotus philippinensis* are used as bitter, anthelmintic, cathartic and styptic.[1] Resin of *Euphorbia resinifera*, a native of Morocco, is used as purgative and abortifacient and in sciatica[1]. The fruits of *Emblica officinalis* is used as acrid, cooling, refrigerant, diuretic and laxative.[1] Fruit is a rich source of vitamin C and is used in the treatment of human scurvy. Such purgative and irritant activity[1] has been ascribed to almost all of the plants given in Table I.

In 1941, the irritant properties of croton oil led Berenblum[2] to detect the augmentational and cocarcinogenic effect of the oil in turmogenesis of mouse skin induced by carcinogenic aromatic hydrocarbons. Berenblum and Shubik[3] applied to the skin of mice

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one single subcarcinogenic dose of 7, 12-dimethyl-benz *a*-anthracene (a carcinogenic aromatic hydrocarbon), which did not elicit any tumors. Also repeated application of such doses of croton oil had no tumorgenic effect. However, a large number of skin tumor is produced by sequential application of the same doses of these compounds if the carcinogen is administered first and the cocarcinogen subsequently, or if the carcinogen dose is administered throughout. In this way, co-carcinogenic activity of croton oil was established.

EXPERIMENT	EXPOSURE OF ANIMALS					TUMORS PRODUCED
1	AR	AR	AR	AR	AR	+
2	AR-	-	-	-	-	-
3	CO	CO	CO	CO	CO	-
4	AR	CO	CO	CO	CO	+

AR = Carcinogenic Aromatic Hydrocarbon

CO = Co-carcinogenic activity of Croton oil

Hecker and co-workers[4] at the German Cancer Research Centre, Heidelberg, West Germany, undertook a systematic fractionation of croton oil, according to the procedure given in table II and followed by measurement of irritant and co-carcinogenic activity and succeeded in isolating 11 compounds from the hydrophilic portion of the oil. These compounds are diesters of a diterpene known as phorbol. Similar diterpene esters were isolated from *Euphorbia resinifera*[5], which were also found to be irritant and co-carcinogenic. Such compounds have now been found in many plants belonging to the family Euphorbiaceae.[6] Some of the diterpene esters have also shown anti-tumor activity.[7] Recently, related diterpene esters have

been isolated from a number of plants belonging to the family Thymeleaceae.[8]

We have investigated the irritant activity of the following plants, which are abundantly available in Pakistan:

1. *Euphorbia caducifolia*
2. *Euphorbia cornigera*
3. *Euphorbia wallichii*
4. *Mallotus philippinensis*
5. *Daphne oleoides*

Except for *Mallotus philippinensis*, all of the plants have shown a high irritant activity.[9] It may be interesting to point out here that whereas in most of the plants studied thus far the irritant activity is located in the hydrophilic fraction,[4] but the hydrophobic fraction of *Daphne oleoides* has been found to be more irritant. Chemical investigations are in progress to isolate these compounds in pure form.

The demonstration of irritant and co-carcinogenic activity by most of the plants belonging to the family Euphorbicaeae suggests that these plants may add to the total carcinogen load of the environment of human beings and provoke certain measures in preventive medicine: that the human beings should be prevented not only from contact with carcinogens, but also from contact with what may be called cocarcinogens such as croton oil.

In view of the above, it is suggested that to begin with, the use of such Euphorbiaceae plants which have shown irritant and co-carcinogenic activity, such as *Croton tiglium* and *Euphorbia resinifera*, should be banned in Islamic medicine. Further investigations should be undertaken on the other plants to assess their suitability for their continued use as drugs.

ACKNOWLEDGEMENT

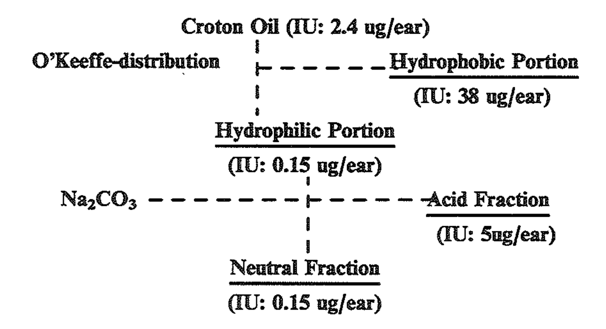
I am indeed grateful to Prof. Dr. E. Hecker, Director, Institute of Biochemistry, German Cancer Research Centre, Heidelberg, Germany for collaborative research programme, International Foundation for Science, Sweden for research grant and the Secretariat, International Organization of Islamic Medicine, Ministry of Public Health, Kuwait for giving me an opportunity to participate in the Conference on Islamic Medicine.

TABLE I: LIST OF EUPHORBIACEAE PLANTS

1. *Acalypha indica*
2. *Aleurites moluccana*
3. *Andrachne cordifolia*
4. *Antidesma bunius*
5. *Baliospermum montanum*
6. *Bischofia javanica*
7. *Breynia patens*
8. *Bridelia montana*
9. *Chrozophora prostrata*
10. *Cicca acida*
11. *Claoxylon indicum*
12. *Cleistanthus colinus*
13. *Codiaeum variegatum*
14. *Croton tiglium*
15. *Drypetes confertiflora*
16. *Emblica officinalis*
17. *Euphorbia species*
18. *Excoecaria acerifolia*
19. *Flueggea leucopyrus*
20. *Hippomane mancinella*
21. *Homonoia rpiaria*
22. *Hura crepitans*
23. *Jatropha curcas*
24. *Macaranga indica*
25. *Mallotus philippinensis*

26. *Manihot esculenta*
27. *Phyllanthus niruri*
28. *Putranjiva roxburghii*
29. *Ricinus communis*
30. *Sapium indicum*
31. *Sauropus quadrangularis*
32. *Sebastinia chamaelea*
33. *Tragia involucrata*
34. *Trewia nudiflora*

Table II: FRACTIONATION OF CROTON OIL



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